Acute inhalation of cigarette smoke increases lower respiratory tract nitric oxide concentrations

D C Chambers, W S Tunnicliffe, J G Ayres

Abstract

Background—Cigarette smoking is associated with a number of common pulmonary diseases including chronic airflow limitation and bronchial carcinoma. Lower respiratory tract (LRT) nitric oxide (NO) concentrations are reduced in habitual cigarette smokers between cigarettes, and although this finding has been implicated in the pathogenesis of smoking related disease, the underlying mechanisms are unclear. A study was undertaken to determine the nature and time course for changes in LRT NO concentrations following acute inhalation of cigarette smoke.

Methods—Twenty four healthy habitual smokers were studied. The concentration of LRT NO in exhaled breath before, one and ten minutes after smoking a single cigarette was measured using chemiluminescence.

Results—LRT NO concentrations increased in all subjects from a mean (SE) of 2.6 (0.27) to 4.8 (0.26) ppb (p<0.0001) at one minute, and at 10 minutes remained significantly raised above the baseline level at 3.2 (0.25) ppb (p = 0.003). The mean (95% CI) increases in NO concentrations were 2.2 (1.7 to 2.7) and 0.6 (0.2 to 1.0) ppb, respectively.

Conclusions—These findings were unexpected in both their direction and time course. They suggest a novel mechanism for the handling of NO in the human lung. We hypothesise that NO is trapped in the epithelial lining fluid (ELF) of the normal human respiratory tract in bioequivalent forms such as S-nitrosothiols or peroxynitrite and that this trapping mechanism is sensitive to the redox state of the ELF. LRT NO concentrations will thus increase with oxidant exposure and decline as pulmonary antioxidant defence mechanisms take effect. These findings may have implications for the pathogenesis and diagnosis of oxidant mediated pulmonary disease.

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Keywords: cigarette smoke; nitric oxide; lower respiratory tract

Nitrile oxide (NO) is produced by the human lung and is involved in the control of the pulmonary circulation and the matching of ventilation with perfusion. NO is also essential for resistance to some pulmonary pathogens. Cigarette smoking is associated with increased susceptibility to colonisation with pulmonary pathogens and with a wide range of pulmonary diseases including chronic airflow limitation and bronchial carcinoma. Many investigators, including ourselves, have reported reduced lower respiratory tract (LRT) NO concentrations in habitual cigarette smokers between cigarettes and concentrations have been shown to increase on cessation of smoking. These findings have been implicated in the pathogenesis of smoking related disease. Although a number of mechanisms have been postulated, it remains unclear how cigarette smoking exerts its effect on LRT NO concentrations. Cigarette smoke contains high concentrations of NO (74.5–1008 parts per million (ppm)) which may directly inhibit nitric oxide synthase (NOS), the enzyme which catalyses the formation of NO from L-arginine. Alternatively, cigarette smoke may inhibit NO production at the level of NOS gene expression through as yet unidentified means. We sought to characterise the nature and time course of the effect of acute inhalation of cigarette smoke on lower respiratory tract NO concentrations in human subjects.

Methods

Regular smokers with no history of respiratory disease were selected to participate in the study. None had suffered upper respiratory tract symptoms in the previous week or were taking inhaled or oral glucocorticoid medication. Subjects with spirometric evidence of airflow obstruction were excluded. All subjects were requested to refrain from smoking for at least one hour prior to taking part.

LRT NO concentrations were measured using chemiluminescence (LR2000, Logan Research, Rochester, Kent, UK) as previously described. Briefly, subjects inhaled to total lung capacity and then exhaled their entire vital capacity through a resistance with a flow meter in series. A visual feedback display allowed the subject to maintain a constant flow rate of 200 ml/s during the exhalation, while the resistance maintained soft palate closure. Nose clips were not used as these may encourage soft palate opening and therefore nasal contamination of the exhalate. The chemiluminescence analyser sampled the exhalate in real time at 250 ml/min (4.2 ml/s) with a sensitivity of 0.3 parts per billion (ppb) and a sampling rate of 25 Hz. Calibration was performed using a standard gas mix. The real time single breath trace thus obtained typically consists of an initial peak followed by a plateau. The LRT NO concentration was obtained from the plateau phase at 75% of exhaled volume.
After assessing the baseline LRT NO concentration subjects smoked one of their usual cigarettes. One minute and 10 minutes after their last puff the LRT NO concentration was reassessed using the same technique.

Data are expressed as mean (SE) unless otherwise specified. 95% confidence intervals were calculated for the mean changes in LRT NO concentration. Comparisons between LRT NO concentrations were made using Student’s paired t tests.

Results
Twenty four subjects (13 women) of median age 29 years (range 21–47) were studied. They had smoked a mean (SD) of 11.5 (11) pack years. The mean (SD) forced expiratory volume in one second (FEV1) was 95.3 (7.8)% predicted and forced vital capacity (FVC) was 96.7 (8.5)% predicted. At baseline the mean LRT NO concentration was 2.6 (0.27) ppb. All subjects had an increase in LRT NO concentration at one minute to a mean of 4.8 (0.26) ppb (p<0.0001), and levels were still significantly higher than the baseline value at 10 minutes (3.20 (0.25) ppb; p = 0.003). The mean (95% CI) increases in NO concentrations were 2.2 (1.7 to 2.7) ppb and 0.6 (0.2 to 1.0) ppb, respectively. The results are displayed in fig 1. In three subjects later measurements were made and the LRT NO concentration had returned to baseline within 20 minutes.

Discussion
We have shown small but highly consistent increases in the LRT NO concentration as a result of smoking a cigarette. These findings were unexpected in both their direction and time course given our current understanding of the cellular and molecular handling of NO in the lung. The LRT NO concentration is reduced between cigarettes in habitual smokers and in vitro studies have shown that cigarette smoke down regulates NO production.\(^{13}\) Kharitonov et al\(^{12}\) when assessing peak NO concentration in the exhaled breath (a method now not used as it may include contaminating nasopharyngeal NO), found a reduced LRT NO concentration five minutes after smoking a cigarette in a subset of the smokers in their study, and postulated that cigarette smoke might inhibit NOS activity or expression. We, in contrast, have observed a universally consistent increase in LRT NO concentrations 1–10 minutes after smoking a cigarette.

There are a number of possible explanations for these findings. First, this could simply represent washout of exogenous NO from cigarette smoke. A simple calculation excludes this possibility. The half life of NO in the LRT is of the order of two seconds\(^{17}\) so, if the starting LRT NO concentration is 1000 ppm (higher than most estimates of the NO content of cigarette smoke\(^{9–11}\)), after 60 seconds (30 half lives) the LRT NO concentration will have fallen to only 0.001 ppb. Second, cigarette smoke may alter NOS expression or activity. Cigarette smoke is known to inhibit NO production in vitro, probably through decreased NOS protein expression.\(^{6–13}\) This effect is both opposite in direction and inconsistent with the time course of our observations as changes in protein expression occur over many hours.\(^{15–16}\) Cigarette smoke is the most potent common oxidant challenge encountered by the human respiratory tract.\(^{17}\) Oxidant stress applied to human pulmonary epithelial cells induces NOS expression through an NF\(\kappa\)B dependent mechanism\(^{14,15}\); however, maximal induction takes hours, reinforcing that induction of gene expression is unlikely to account for our observed increase in LRT NO concentrations. NOS enzyme activity has been shown to increase rapidly on delivery of substrate (l-arginine)\(^{15,22}\) and to decline with hypoxia\(^{11}\) and in the presence of specific NOS inhibitors such as N\(^{-}\)-monomethyl-l-arginine and aminoguanidine. Both carbon monoxide (CO)\(^{22}\) and NO\(^{12}\) inhibit NOS through binding to its haeme subunit, but no component of cigarette smoke is known to increase NOS activity.

A third possible explanation concerns the affinity of NO for haeme.\(^{22}\) The LRT NO concentration is proportional to the rate of production of NO and inversely proportional to its transfer factor (TLNO).\(^{23}\) The TLNO is partly dependent on the reaction of NO with haeme\(^{15}\) so a decreased availability of haeme could result in increased LRT NO concentration. CO can compete with NO for haeme if present in high concentrations,\(^{25}\) but we found no change in the LRT NO concentration after exposing subjects to higher concentrations of CO than those encountered during smoking (data not shown). An effective decrease in TLNO occurs if NO is unavailable for reaction with haeme. The chemistry of the oxides of nitrogen suggests that NO is likely to react before being taken up in the pulmonary capillary. Superoxide (O\(_2\)\(^-\)) reacts very rapidly with NO to form peroxynitrite (ONOO\(^-\)), a reaction facilitated by the acidic pH of the epithelial lining fluid (ELF) and the O\(_2\) rich environment of the lung.\(^{26}\)
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O\textsuperscript{2−} + NO ⇔ OONO\textsuperscript{−} ⇔ OONOH ⇔ NO\textsuperscript{2−} + H\textsuperscript{+}.

 Peroxynitrite and peroxynitric acid (OONO\textsuperscript{−}) are both potent oxidising agents and are microbicidal and cytotoxic. The thiols containing antioxidants glutathione and albumin are found in high concentrations in the ELF\textsuperscript{[27]} and are nitrosated by NO redox equivalents (N\textsubscript{2}O\textsubscript{3} and N\textsubscript{2}O\textsubscript{5}) to produce S-nitrosothiols (RS-NO).\textsuperscript{[25]}

S-nitrosothiols are much less reactive than free NO, but retain NO-like biological activity.\textsuperscript{[28]} It is likely that the “trapping” of high local concentrations of these more stable bioequivalents through redox cycling is important in the pulmonary physiology of NO and may be a highly efficient mechanism by which the oxides of nitrogen modulate their biological effects.\textsuperscript{[29]}

We hypothesise that the strong oxidant challenge presented to the lung by cigarette smoke leads to locally oxidant conditions in the ELF, and through a direct chemical effect increases concentrations of NO in the LRT. Oxidant stress may decrease the availability of reduced thiol compounds, may increase decomposition of nitrosothiols, or may shunt NO away from haeme by cycling through redox equivalents. More work is necessary to identify the responsible oxidents in cigarette smoke and to elucidate the complex chemistry within the ELF, but it may be that the magnitude of the change in the LRT NO concentration following acute oxidant stress reflects the pulmonary antioxidant capacity and that the time course of the change reflects the efficiency of mechanisms to restore this capacity.

Habitual smokers have unusually high antioxidant concentrations in the ELF and a reduced sensitivity to ozone induced pulmonary damage.\textsuperscript{[27][29][30]} This may reflect an adaptive response to chronic oxidant stress and may also explain the reduced LRT NO concentrations observed in habitual smokers between cigarettes.\textsuperscript{[3][4][7]} Asthmatic inflammation is associated with marked increases in LRT NO concentrations,\textsuperscript{[1][11]} most probably due to induction of NOS. However, the oxidant stress imposed on the airway by this condition may further increase NO concentrations. In summary, we suggest that NO is “trapped” at the epithelial surface of the normal human LRT in the form of bioequivalent oxides of nitrogen such as peroxynitrite and S-nitrosothiols, a process central to NO pulmonary physiology, and that this trapping mechanism will be redox sensitive. The LRT NO concentration should therefore at least partially reflect LRT redox conditions. We further suggest that oxidant stress increases the LRT NO concentration and that the chronically reduced state of the ELF in habitual cigarette smokers is reflected in a decreased LRT NO concentration between cigarettes. Further study is necessary to determine whether the response of LRT NO concentrations to acute oxidant stress may be a useful measure of pulmonary antioxidant capacity and therefore of susceptibility to oxidant mediated pulmonary disease.

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