Published in final edited form as:

Ann Otol Rhinol Laryngol. 2011 July ; 120(7): 455–459.

Nasal Nitric Oxide and Lifestyle Exposure to Tobacco Smoke

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Abstract

Objectives—Nitric oxide (NO) is a reactive gas generated by inflammatory cells and mucosal epithelial cells of the nose and paranasal sinuses and is an important mediator in nonspecific host defense against infectious agents. However, NO also mediates physiologic events such as vasodilation, mucus hypersecretion, and mucosal disruption that are associated with inflammatory conditions, and it is a regulator of ciliary beat frequency. In the present study, we hypothesized that lifestyle exposure to tobacco smoke, whether through active smoking or by inadvertent exposure to secondhand tobacco smoke, would result in higher detectable levels of nasal NO (nNO) than are found in well-documented nonsmokers.

Methods—Nasal NO measurements were obtained concomitant with assays of urine cotinine from well-documented nonsmokers, active smokers, and individuals exposed by lifestyle to secondhand smoke. These parameters were statistically analyzed to determine whether increasing levels of tobacco smoke exposure yield higher concentrations of nNO.

Results—Our results and subsequent statistical analyses imply that active smokers who exhibit high urine cotinine levels exhibit significant increases in nNO levels in comparison to both nonsmokers and nonsmokers exposed to secondhand smoke.

Conclusions—There is an increased level of nNO associated with tobacco smoke exposure that may contribute to the inflammatory processes characteristic of disease pathogenesis in smokers.

Keywords

inflammation; nitric oxide; secondhand smoke; tobacco smoke

INTRODUCTION

Since the discovery of its function as the endothelium-derived relaxing factor,¹ nitric oxide (NO) has been further documented to be an important physiologic signaling molecule, to have proinflammatory properties, and to undergo detectable modifications of concentration in certain pathologic conditions. The development of sensitive techniques for instantaneous measurement and monitoring of NO concentration in human airways spurred many clinical studies in which NO excretion into the nasal and lower airways was related to a variety of diseases, as well as to therapeutic interventions. These discoveries have led to speculation on the potential of NO as a biomarker of health and disease. For example, extensive studies have strongly suggested that NO flux measurements may have potential clinical utility in the

management of asthma^{2,3} or as a potential diagnostic marker of rare airway diseases such as cystic fibrosis⁴ and primary ciliary dyskinesia.⁵

The NO in the airways is synthesized from L-arginine and oxygen by three nitric oxide synthase (NOS) isoforms.^{6–8} Although airway epithelial cells clearly express NOS, produce NO, and are believed to be the major source of lower airway NO, it is less clear which NOS isoforms are expressed under conditions of normal function or disease at different levels of the airways. There exists a particular deficit of information regarding nasal NO (nNO) levels and tobacco smoke exposure. Thus, the lack of a clear understanding of the relationship between NO excreted into the airway lumen, total NO production, and the role of the constitutive NOS isoforms in different pathologic states has reduced the ability to interpret the quantitative data provided by this emergent noninvasive marker of airway disease. In the present study, we assessed nNO levels among human subjects with a variety of lifestyle tobacco smoke exposure histories and correlated them with urine cotinine values.

METHODS

Human Subjects

This study was reviewed and approved by The University of North Carolina at Chapel Hill Office of Human Research Ethics. A total of 88 subjects with a broad range of smoking histories were recruited. At the time of recruitment, all of the subjects, regardless of smoking history, reported subjectively that their overall health was good. Asthma and/or other previously diagnosed chronic respiratory diseases were exclusion criteria. Participants self-reported their status upon entering the study as belonging to 1 of 3 groups: 1) ex-smokers and nonsmokers, 2) current smokers, and 3) nonsmokers who are domestically or occupationally exposed to secondhand tobacco smoke (NS/SHS). The individuals who identified themselves as smokers subjectively reported recent cigarette use as no more than 2 packs of cigarettes per day. The subjects also completed a questionnaire assessing their general health, current respiratory symptoms, and other relevant information that allowed consideration for exclusion from the study if indicated. There were 35 nonsmokers, 35 smokers, and 18 NS/SHS in the study groups. There were 38 male and 50 female subjects, and the race distribution was consistent with the population demographics of central North Carolina. The median ages were 25 years for nonsmokers, 34 years for smokers, and 26 years for NS/SHS.

Nasal Nitric Oxide Assays

The nNO level was measured by use of a Sievers model 280i NOA nitric oxide analyzer (General Electric Analytical Instruments, Boulder, Colorado) employing a mouth resistor method.⁹ The disposable nasal sampling probes and mouth resistors were obtained from DirectMed, Inc (Glen Cove, New York). The sampling flow rate was 0.5 L/min. The nNO determination protocol was based on American Thoracic Society/European Respiratory Society recommendations.⁹ Briefly, after explanation of the procedure to the subject, a nasal probe connected to the sampling line and the analyzer was inserted into a nostril. Afterward, the subject was asked to inhale fully and subsequently exhale slowly through a mouth resistor. The nNO signal was displayed on a monitor, and once the nNO plateau was reached and maintained for at least 10 seconds, the acquisition was stopped. The measurement was repeated until 2 acceptable curves were obtained for each nostril (2 or 3 trials). The nNO concentration (in parts per billion) for each trial was analyzed over a 3- to 5-second interval of a plateau section of the curve. Nasal NO concentrations were acquired from each nostril, and the values for both nostrils were averaged and converted into nNO production in nanoliters per minute.

Cotinine Assays

Each study subject provided a urine specimen coordinated with the time of the nasal sampling to obtain a quantitative measure of tobacco smoke exposure and to validate self-reported tobacco exposure histories. No further effort was made to subcategorize tobacco exposure levels in the different groups; rather, each group served to provide a range of lifestyle exposure levels as a function of the type of exposure. Cotinine analyses were performed with a commercially available enzyme-linked immunosorbent assay (Bio-Quant, San Diego, California). Creatinine analyses were performed with a colorimetric assay (Oxford Biomedical Research, Oxford, Michigan). From these data, a cotinine-creatinine ratio (CCR) was determined for each subject. Because of the great variability in values across the spectrum of exposure, the CCR values were converted to log 10 for presentation purposes.

Statistical Analyses

A simple summary of descriptive statistics of the data is given in Table 1. The graph in Fig 1, fitted with a crude local polynomial fitting (LOESS),^{10,11} depicts an overall and unadjusted relationship between nNO level and CCR. To investigate the association between nNO level and CCR and smoking status, we fitted a linear model in which nNO level was the outcome variable and CCR and smoking status were the independent variables. We first fitted an oversaturated model (full model) that included age, gender, CCR, CCR², smoking status, and interaction terms between CCR and smoking. We then used the likelihood ratio method to remove factors that were not statistically significant in the model. We arrived at the final model that was deemed by data and statistical hypothesis testing to best describe the relationship. To determine whether the nNO level is related to smoking status, conditional on the subject's age, gender, and CCR, the regression analysis is the best statistical approach to optimally analyze the data. This approach to analysis also complements the hypothesis relative to the NO physiology in the upper airways,⁶⁻⁸; inasmuch as NO is produced by both the nasal and paranasal sinus mucosa, as well as by inflammatory cells, and is a known regulator of ciliary beat frequency (CBF), and it is plausible that production may be linked to inflammatory agents such as tobacco smoke.

RESULTS

Descriptive statistics (Table 1) of relative nNO and CCR values revealed little evident difference between nonsmokers and NS/SHS. In contrast, nNO levels were reduced among smokers relative to nonsmokers and NS/SHS, and there was marked variability in individual measurements. As expected, the CCR was dramatically higher among smokers than in either of the nonsmoker groups. An unadjusted association between nNO and CCR values was identified by local polynomial fitting (LOESS; Fig 1). Figure 1 suggests that there might be a nonlinear relationship between nNO level and CCR. A rigorous test from our regression analysis results revealed there was a significant quadratic relationship between CCR and nNO level ($p = 0.008$) and that there was a significant smoking group effect, with smokers as the reference ($p = 0.008$ for nonsmokers and $p = 0.006$ for NS/SHS). The final model fit is presented in Table 2. Age and gender showed no effect on nNO level ($p = 0.5$ for age and $p = 0.41$ for gender). We also found a marginally significant interaction effect ($p = 0.06$) between smoking status and CCR (not presented), but this effect may require a larger data set to confirm. Figure 2 illustrates the final fitted regression model demonstrating that among smokers, a positive relationship exists between nNO level and CCR, whereas both nonsmokers and NS/SHS exhibited a comparable flat-to-negative association between nNO level and CCR (Fig 2). Among smokers exhibiting higher CCR values, the relationship between an elevated CCR and an increased nNO level was dramatic.

DISCUSSION

Tobacco smoke exposure elicits an inflammatory response,^{12,13} and both the airway mucosa⁷ and the inflammatory cells¹⁴ contain NOS. Thus, we initially hypothesized that nNO levels would be elevated uniformly among smoke-exposed individuals relative to nonsmokers. This, however, was not the case, and instead we observed great variability in individual measurements of nNO among active smokers and an overall reduction in nNO level among active smokers relative to nonsmokers and NS/SHS. In addition to an inflammatory response, smokers may typically exhibit nasal congestion and rhinorrhea, factors that may be in part responsible for the variability of nNO measurements among active smokers in this study. Not only may nasal congestion occlude the paranasal sinuses, an anatomic site of relatively higher NO production, but NO generated from the mucosa, being highly reactive, may interact with secretions to reduce the nNO level. Conversely, the excessively high nNO levels that were observed among some subjects may be reflective of relatively unobstructed airways and tobacco smoke-induced increases in nNO.

Mucociliary clearance is mediated in part by the ability of the airway mucosa to up-regulate CBF through an NO pathway.^{15,16} Other studies have suggested that regulation of ciliary function exhibits both adrenergic and cholinergic components.^{17–19} Moreover, irritants in ambient air, including tobacco smoke,²⁰ have been shown to accelerate the CBF. Previous studies from this laboratory²¹ demonstrated a generalized up-regulation of CBF among human subjects caused by active smoking and passive exposure. In vitro investigation of this phenomenon using an NO-specific fluorescent dye in cultured cells suggested that NO is generated in airway epithelial cells at a level above background by exposure to cigarette smoke condensate.

Nitric oxide is highly reactive and is capable of many chemical interactions with cellular constituents, particularly tyrosine²² — a capability that may account for the reduced level of nNO among some smokers. Previous studies of smokers and subjects with asthma also have found reduced exhaled NO levels accompanied by increased numbers of mucus-producing cells. This situation may produce a rich chemical milieu in the airway surface liquid that facilitates interactions with NO, thereby reducing its concentration upon excretion.^{23,24} In this study, the highest levels of nNO were observed in smokers, who exhibited an 8.6-fold difference between the maximum and minimum nNO values, in contrast to nonsmokers and NS/SHS, who exhibited 2.7- and 3.8-fold differences, respectively. This great variability in nNO measurements among smokers suggests that other factors may mitigate NO interaction with cellular constituents and secretions among smoking subjects, thereby resulting in readings of elevated NO levels. Although mucociliary clearance causes a net transport of secretions out of the airway, it is plausible that accumulation and clearance of the airway surface liquid exhibits some regional variability that may either limit or promote the interaction of the airway surface liquid with NO.

The biphasic model suggests that relative to nNO level and CCR, the NO physiology among smokers differs from that of nonsmokers and NS/SHS. The fact that the study population consisted of several subpopulations (strata) that behave differently in terms of the nNO-CCR relationship is best distinguished by the linear regression analysis.

In summary, we have demonstrated an association between increased nNO levels as a function of active smoking and CCRs measured during the same time interval. This association is most pronounced at higher CCRs, suggesting that heavy smokers excrete more NO than light smokers. In contrast, urine cotinine levels of nonsmokers and subjects exposed by lifestyle to secondhand tobacco smoke exhibited no significant association between exposure and nasal NO levels. In summary, the cell molecular interactions of NO in

the upper airways in response to tobacco smoke exposure are likely multifactorial, being on one hand elevated and contributing to the inflammatory response and regulation of ciliary function, and being on the other hand limited by interaction with cellular proteins and excess secretions associated with irritant exposure.

Acknowledgments

The authors thank Sally Ivins for coordination of subject recruitment, Lynne Newlin-Clapp, Martha Almond, and Carol Robinette for technical assistance with nasal nitric oxide determinations, and Margaret Herbst for administrative assistance.

This study was supported by grant R01CA79949 from the National Institutes of Health (H.Z.), a Clinical Innovator Award from the Flight Attendant Medical Research Institute (J.L.C.), and the US Environmental Protection Agency. Although the research described in this article has been funded wholly or in part by the US Environmental Protection Agency through cooperative agreement CR833463-01 with the Center for Environmental Medicine, Asthma, and Lung Biology at the University of North Carolina at Chapel Hill, it has not been subjected to the agency's required peer and policy review, and therefore does not necessarily reflect the views of the agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Fig 1. Unadjusted association between nasal nitric oxide and cotinine-creatinine ratio values via local polynomial fitting (LOESS). NS/SHS — nonsmoker who is domestically or occupationally exposed to secondhand tobacco smoke.

Fig 2.
Association between nasal nitric oxide and cotinine-creatinine ratio values based on final regression model for 30-year-old (mean age) male subject.

TABLE 1

Summary statistics of nNO levels and CCR among human subjects

Category	Sample Size	nNO (mean nL/min $\pm$ SD)	CCR* (mean $\pm$ SD)
Nonsmokers	35	303 $\pm$ 90	1.06 $\pm$ 0.77
NS/SHS	18	304 $\pm$ 88	1.34 $\pm$ 0.78
Smokers	35	278 $\pm$ 136	5.43 $\pm$ 1.46

nNO — nasal nitric oxide; CCR — cotinine-creatinine ratio; NS/SHS — nonsmokers who are domestically or occupationally exposed to secondhand tobacco smoke.

* log₁₀.

TABLE 2

Regression analysis results for nNO

Parameter	Estimate	SE	P
Intercept	215.09	64.40	0.001
CCR	-33.46	24.64	0.178
CCR ²	8.94	3.30	0.008
Age	-0.93	1.30	0.477
Gender (female)	-20.94	25.02	0.405
NS/SHS	158.01	56.04	0.006
Nonsmokers	146.56	53.66	0.008

Male is reference for gender, and “smokers” is reference for smoking status. Regression model: $nNO = \alpha + \beta_1 CCR + \beta_2 CCR^2 + \beta_3 Age + \beta_4 I(\text{gender} = \text{female}) + \beta_5 I(\text{smoking} = \text{NS/SHS}) + \beta_6 I(\text{smoking} = \text{nonsmokers}) + \epsilon$, where $\epsilon \sim N(0, \sigma^2)$.