Non-invasive method of measuring airway inflammation: exhaled nitric oxide

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Abstract

Background

Nitric oxide (NO) was well known to be a component of air pollution, often in the form of nitrogen dioxide (NO_2). However its importance in biological systems altered dramatically with the discovery in 1987 that it was the 'endothelial-derived relaxing factor'. Since then there has been an explosion of research on NO demonstrating that this gaseous molecule was a widespread physiological mediator and was simultaneously recognised as a vital component of immune function contributing to macrophage-mediated cytotoxicity. NO was therefore a key molecule in modulating inflammation, including airway inflammation.

The aim of this thesis was:

1. To adapt a NO chemiluminscence analyser from measuring airway pollution to measuring exhaled air in human subjects.
2. To measure NO levels in exhaled air in adult subjects.
3. To evaluate whether altering measurement parameters altered the levels of NO obtained.
4. To adapt this technique from adults to measure exhaled NO in children.
5. To compare levels of NO from healthy children to groups of asthmatic children on either bronchodilator therapy only, or on regular inhaled corticosteroids.
6. To compare the levels of NO in a pilot group of asthmatic children before and after commencement of inhaled corticosteroids.

Methods

A Dasibi Environmental Corporation Model 2107 chemiluminescence analyser was adapted specifically requiring a reduction in response time, which was achieved by modification of the circuitry and re-routing of the analogue signal directly to a chart recorder, achieving a reduction of the response time by 80%. Addition of a number of analysers allowed the measurement of exhaled NO, carbon dioxide (CO_2), mouth pressure and flow for each exhalation from total lung capacity. Twenty adult subjects (in total) were then studied looking at direct (NO, CO_2, mouth pressure) versus t-piece (with the addition of flow) measurements making five exhalations from total lung capacity, at 3-minute intervals (direct/t-piece/direct or t-piece/direct/t-piece in series). The area of NO under the curve versus the peak of the NO trace was compared and the exhalation pattern of NO versus CO_2 was compared. Measurement conditions were altered to evaluate the effect of individual parameters on the exhaled NO result. This included separately assessing different expiratory flows, different expiratory mouth pressures, the effect of a high versus a low background NO level and the

effect of drinking water (of varying temperatures) prior to exhalation. Healthy control children were then enrolled to the study from a local school (Park Walk Primary School) and compared with asthmatic children enrolled from outpatient clinics at the Royal Brompton Hospital. The asthmatic children were further divided into those on bronchodilator treatment only and those on regular inhaled corticosteroid therapy. NO was also measured before and two weeks after commencing inhaled corticosteroid therapy in previously steroid-naive asthmatics.

Results

It was possible to modify a chemiluminescence analyser to enable measurement of exhaled NO. In 12 healthy subjects (mean age 32 years, 6 males) peak direct NO levels were 84.8 parts per billion (ppb) (standard error of the mean (SEM) 14.0ppb), significantly higher than 41.2ppb (SEM 10.8ppb) measured via the t-piece system. The exhaled NO rose to an early peak and plateau while the CO_2 levels continued to rise to peak late in the exhalation. The mean times to peak NO levels were 32.2 seconds (s) (direct) and 23.1s (t-piece), which was significantly different from the mean times to peak CO_2 levels at 50.5s (direct) and 51.4s (t-piece, $p<0.001$). At peak NO level, the simultaneous CO_2 level of 4.9% (SEM 0.14%, direct) and 5.2% (SEM 0.18, t-piece) were significantly lower than the peak CO_2 achieved of 5.8% (SEM 0.21%, direct, $p< 0.001$) and 6.2% (SEM 0.28, t-piece, $p<0.001$). There was no difference between repeat direct or t-piece measurements.

With regard to varying measurement conditions, the mean peak concentrations of NO decreased by 35ppb (95% confidence intervals 25.7-43.4) from a mean of 79ppb (SEM 15.4ppb) at an expiratory flow rate of 250mls/min to 54.1ppb (SEM 10.7ppb) at 1100mls/min. The mean peak concentration of NO did not change significantly when mouth pressure was increased in eight of ten subjects, although in two it did decrease in the highest pressure. The mean NO concentration with machine and subjects sampling from a low NO reservoir was 123ppb (SEM 19.4), which was an increase from results obtained before at 81.9ppb, SEM 10.2ppb, p=0.001 95%, CI -19.9 to -62.7) and after at 94.2ppb (SEM 18.3ppb, p=0.017, 95% CI 6.0-5.18) sampling with high ambient NO levels. The mean peak NO concentration decreased from 94.4ppb (SEM 20.8) to 70.8ppb (SEM 16.5, p=0.002 95% CI 12.9-33.1) with water consumption.

In 39 healthy pre-pubertal children with a mean age of 9.9 years (range 9-11 years, 23 girls) the mean direct exhaled NO level was 49.6ppb (SD 37.8ppb, range 11.5-197.2ppb) compared with mean exhaled NO via t-piece sampling of 29.2ppb (SD 27.1ppb, range 5.1-141.2ppb).

There was no significant difference between boys and girls for either the direct or the t-piece recordings. In comparison with normal children, 15 asthmatic children on bronchodilator therapy only had much higher levels of exhaled NO at 126.1ppb via the direct system (SD 77.1ppb, $p<0.001$) and 109.5ppb via the t-piece system (SD 106.8ppb, $p<0.001$). In 16 asthmatics on regular inhaled corticosteroids the mean peak exhaled levels were significantly lower at 48.7ppb via the direct method (SD 43.3ppb, $p<0.001$) and via the t-piece system at 45.2ppb (SD 45.9ppb, $p<0.01$). There was no difference between the normal children and the asthmatic children who were on regular inhaled corticosteroids ($p=0.9$ direct, $p=0.2$ t-piece). There was no significant difference in CO_2, mouth pressure, duration of expiration and expiratory flows between the three groups or between the two methods (direct and t-piece). In six asthmatic children the mean peak exhaled NO levels fell from a medium peak level of 124.5ppb to 48.6ppb when measured before and two weeks after commencement of inhaled corticosteroids on treatment.

Discussion

This research showed it was possible to modify an NO chemiluminescence analyser to enable measurement of exhaled NO in adult and paediatric subjects. Furthermore, it was possible to measure both healthy and asthmatic children. There were significant differences between the exhalation pattern of NO and CO_2 suggesting that NO was produced in the airways, not at alveolar level, unlike CO_2. The measurement of exhaled NO required a standardised approach as exhaled NO levels decreased with increasing expiratory flow, when measuring at a time of high ambient NO concentration, and with consumption of either hot or cold water immediately preceding exhalation (such as might be given if a subject was coughing). The findings with expiratory mouth pressure were less certain, with a difference seen in only two of ten subjects.

The levels of exhaled NO measured in children aged 9-11 years were lower than that measured in the adult subjects. There was no difference between boys and girls, or with other parameters such as having a personal history of atopy, a family history of atopy, or the presence of a smoker or furry pets within the house-hold. These findings may have altered with increased numbers in this group and could possibly be a type two statistical error. The results of exhaled NO in asthmatic children on bronchodilator therapy only were significantly elevated compared to both normal children and asthmatic children treated with regular inhaled corticosteroids. The exhaled NO level also fell significantly by two weeks following the commencement of inhaled corticosteroid treatment in steroid-naive asthmatic children. These

results suggested that the methods of measuring exhaled NO required standardization and that it could potentially be a non-invasive measure of airway inflammation to follow - particularly in children with asthma who were commencing inhaled steroid treatment.

Dedication

This is Dedicated:

To the strong women in my family from my great, great grandmother

to my sister Angela who always believes in me.

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Contents

List of Tables

Page

List of Figures

Page

List of Abbreviations

<	less than
>	greater than
ABPA	allergic bronchopulmonary dysplasia
ATS	American Thoracic Society
ATP	adenosine triphosphate
BAL	bronchoalveolar lavage
BH_4	tetrahydrobiopterin
BTS	British Thoracic Society
C3, C5	complement factor 3, complement factor 5
cAMP	cyclic adenosine 3', 5' monophosphate
CAT1, CAT2, CAT2B, CAT 3	cationic amino acid proteins
CB	chronic bronchitis
CD3+	cell marker for T lymphocyte
CD4+	cell marker for T helper lymphocyte
CD8+	cell marker for T cytoxic lymphocyte
CF	cystic fibrosis
cGMP	cyclic guanosine monophosphate
cNOS	constituent nitric oxide synthase
CNS	central nervous system
Co	cobalt
CO	carbon monoxide
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
CSF	central spinal fluid
Cu	copper
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECMO	extracorporeal membrane oxygenation
ECP	eosinophil cationic protein
ECRHS	European Community Respiratory Health Survey
EDRF	endothelial derived relaxing factor

ELISA	Enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
EPN/EPX	eosinophilic neuraminadase
EPO	eosinophilic peroxidise
ERS	European Respiratory Society
ERSTF	European Respiratory Society Task Force
FAD	flavin adenosine dinucleotide
Fe	iron
$FEF_{25-75\%}$	forced expiratory flow at 25 to 75 percent of forced vital capacity
FEV_1	forced expiratory volume in 1 second
$FEV_{0.5}$	forced expiratory volume in half a second
FMN	flavin mononucleotide
FVC	forced vital capacity
GINA	Global Initiative for Asthma
GM-CSF	Granulocyte macrophage – colony stimulating factor
GTP	guanosine 5-triphosphate
H_2O	water
H_2S	hydrogen sulphide
HNO_2	nitrous acid
HRCT	high resolution computerised tomography
ICAM1	intracellular adhesion molecule 1
IFNγ	interferon gamma
IgA	immunoglobulin A
IgG	immunoglobulin G
IgE	immunoglobulin E
IHCS	inhaled corticosteroids
IL1, IL2, IL3, IL4, IL5, IL6, IL8, IL9, IL10, IL11, IL12, IL13, IL17	interleukin 1, interleukin 2, interleukin 3, interleukin 4, interleukin 5, interleukin 6, interleukin 8 interleukin 9, interleukin 10, interleukin 11, interleukin 12, interleukin 13, interleukin 17
iNOS	inducible nitric oxide synthase
ISAAC	International Study of Asthma and Allergies in Childhood
Kb	kilobases
L/min	litres per minute
LABA	long acting β_2 agonist

LADA	N^wN^w dimethyl L-arginine
LFA1	lympocyte function-associated antigen 1
L-NAME	N^w arginine methyl ester
L-NMMA	N^w monomethyl L-arginine
L-NOARG	N^w-nitroarginine
LNIO	N- imino-ethyl-ornithine
LPC	lysophosphotidylcholine
LPS	lipopolysaccharide
LTC4, LTD4, LTE4	leukotriene C4, leukotriene D4, leukotriene E4
MBP	major basic protein
mg/ml	milligrams per millilitre
mls/min	millilitres per minute
Mn	manganese
mRNA	messenger ribonucleic acid
N_2	nitrogen
N_2O	nitrous oxide ("laughing gas")
N_2O_3	dihydrogen trioxide
N_2O_4	dihydrogen tetraoxide
NADPH	nicotinamide adenosine di-nucleotide phosphate
NANC	non adrenergic, non cholinergic (nerves)
NF-κB	nuclear factor-kappa B
NHANES 1	National Health and Nutrition Examination Survey 1
nL/min	nanolitres per minute
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NO +	nitrosoium cation
NO-	nitroxyl anion
NO_2	nitrogen dioxide
NO_2-	nitrite
NO_3-	nitrate
NO_2Tyr	3-nitrotyrosine
NOS	nitric oxide synthase/s
NOx	nitrogen oxides (usually NO, NO_2 and NO_3)
NZPAG	New Zealand Paediatric Asthma Guidelines
O_2	oxygen

O_2-	superoxide anion
O_3	ozone
OH-	hydroxyl anion
ONOO-	peroxynitrite
ONOOH	peroxy nitrous acid
PaO_2	pulmonary artery oxygen
PCD	primary ciliary dyskinesia
PC_{20}	provocation concentration producing 20% fall in the forced expiratory volume in one second
PD_{20}	provocation dose producing 20% fall in the forced expiratory volume in one second
PEF	peak expiratory flow
Pmo	mouth pressure
ppb	parts per billion
ppm	parts per million
PM10	particulate matter with a diameter of less than 10μm
redox	reduction oxidative reactions
ROC	receiver-operator curve
RS	sulphur thios
RSV	Respiratory Syncytial Virus
SD	standard deviation
SEM	standard error of the mean
sGC	soluble guanylate cyclase
SIGN	Scottish Intercollegiate Guidelines Network
SLE	systemic lupus erythematosis
SO_2	sulphur dioxide
TB	tuberculosis
TGFβ	transforming growth factor beta
T_H1	T helper lymphocyte cell type 1
T_H2	T helper lymphocyte cell type 2
T_H3	T helper lymphocyte cell type 3
TNFα	tumour necrosis factor alpha
TNFβ	tumour necrosis factor beta
type I (nNOS)	Type I neuronal nitric oxide synthase
type II (iNOS)	Type II induced nitric oxide synthase

type III (eNOS)	Type III endothelial nitric oxide synthase
UK	United Kingdom
μg/ml	micrograms pre millilitre
UNICEF	United Nations International Children's Fund
USA	United States of America
UV light	ultraviolet light
V/Q	ventilation and perfusion ratio
Zn	zinc