Reduced endogenous nitric oxide in the exhaled air of smokers and hypertensives

J. Schilling*, P. Holzer*, M. Guggenbach*, D. Gyurech**, K. Marathia*, S. Geroulanos*

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ABSTRACT: We wanted to determine whether the production of endogenous nitric oxide (NO) is affected by cigarette smoking and various pathological conditions.

Endogenously produced NO was measured by chemiluminescence in the exhaled air of 81 healthy volunteers (21 nonsmoking females (NSF), 12 smoking females (SF), 24 nonsmoking males (NSM) and 24 smoking males (SM)) and 38 patients (10 with hypertension, 10 intra- and 10 postoperative, 5 with renal failure and 3 with sepsis) entered the protocol. Subjects inspired from a NO-free air supply, which was also used to calibrate the NO-analyser.

Endogenous NO production of volunteers was 18 ± 8 per billion (ppb) depending on smoking habits. In exhaled air of NSF, NO concentration was 21 ± 7 ppb, in SF 16 ± 6 ppb, in NSM 19 ± 8 ppb and in SM 15 ± 6 ppb. Differences between smokers and nonsmokers were significant. Increased diastolic blood pressure was noted in SM compared to NSM (86 ± 7 versus 78 ± 7 mmHg). Patients with documented and treated hypertension (systolic and diastolic blood pressure: 141 ± 18 and 82 ± 9 mmHg) exhaled 13.7 ± 5.3 ppb NO; hypertensive males 10 ± 2 ppb NO and females 17 ± 5 ppb NO. In patients with renal failure NO concentration in exhaled air was 20.2 ± 6.8 ppb before and 19.8 ± 6.4 ppb one hour after the onset of dialysis. In patients undergoing major surgery NO concentration was 5.6 ± 2.5 ppb intra- and 10.3 ± 3.5 ppb postoperatively. In three mechanically ventilated patients with documented septic syndrome, exhaled NO was 29.3 ± 24 ppb.

We conclude that smokers and patients with hypertension exhale significantly less NO than healthy volunteers. This suggests that exhaled NO can be used as a marker and therapeutic target in disease.

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Depts of *Surgery and **Social and Preventive Medicine, University Hospital of Zurich, Zurich, Switzerland.

Correspondence: S. Geroulanos
Dept of Surgical Intensive Care
Onassis Center
Leof. Syngrou Av. 356
GR - 176 74 Kallithea
Athens

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Nitric oxide acts as an endogenous vasodilator molecule [1–4]. It is synthesized from L-arginine, largely by the "organ endothelium" (6×10^{23} cells, 1,000 square meters surface, 1.5 kg weight) and various other tissues, as well as cells in the alveoli and airways [1, 5]. Its plasma half-life is only a few seconds [6, 7].

As NO acts as an endogenous gas-vasodilator [1–4] it is possible that it is involved in the physiological control of systemic blood pressure. For this reason, methods to assess endogenously produced NO are required. Currently, no blood gas analysers for NO are available. Exhaled air may be a logical medium to evaluate endogenously produced NO, although exhaled air-concentrations may not reflect systemic blood concentrations, due to its reactivity and local lung production.

The aim of this study was to evaluate endogenously produced NO in exhaled air, focusing on differences between several population groups. In contrast to other studies [8], subjects inspired from a NO-free air supply, as ambient air is contaminated with various amounts of NO.

Materials and methods

In this cohort trial, endogenously produced NO was measured by chemiluminescence in the expired air of 119 subjects. As ambient air contains a high and variable NO concentration (6–192 ppb) a standardized, purified NO, NO₂, CO, CO₂ and ozone free air was prepared for inhalation by our study population. Under a constant pressure, normal hospital air passed a water filter, a CO-CO₂ converter (heated palladium), a purafil-converter (granular aluminium oxide and potassium permanganate) to oxidize NO to NO₂ and finally an active-coal filter to absorb CO₂ and NO₂ molecules. The purified gas mixture was accumulated in flexible, empty polyethylene reservoirs and analysed for NO after production and immediately before use. An upper limit of up to 5 ppb NO was accepted. For all NO analyses, the commercial instrument CLD 700 AL (ECO Physics, 8635 Dürnten, CH) was utilized. Calibration was performed weekly using a diluted calibration-gas-mixture of exactly 900 ppb. Fifty to 100 parts per million (ppm) NO were

diluted with a dry, NO, NO₂, CO, CO₂ and ozone free N₂O₂ mixture (1:100–500). For calibration only Teflon-coated tube-systems were used. The minimal detectable NO concentration was 1 ppb, corresponding to 0.000,0001 volume-percent. Calibration and all measurements were performed in the same chamber under constant conditions.

Preliminary experiments

To determinate whether the polyethylene material of the specially manufactured reservoirs and tube systems interferes with NO measurements, three tubes and three reservoirs were filled with 5 ppb NO. After 6 and 12 h, NO concentration was still 5 ppb in the tubes and reservoirs, and after 24 h 8 ppb in two of the reservoirs. The same experiment was performed with 30 ppb, with similar results (± 2 ppb). In a third experiment, a reservoir was filled with 16 ppb NO at a temperature of 26°C, heated once to 40°C and cooled down once to 12°C for 30 min. In these experiments, no changes were observed. In a fourth experiment, the role of the diameter and length of inspiratory and expiratory tubes were evaluated. We found no differences in the NO concentration using tubes up to 2 m long and up to 3 cm wide. In addition, compressed standard hospital-room-air (N₂/O₂) was analysed. NO concentration varied over 7 days (27.4 $\pm$ 13 ppb), depending on the weather and possibly on the traffic conditions in the vicinity of the hospital. Mean NO concentration of normal hospital-room-air was 54 $\pm$ 58 ppb, with a minimum of 6 and a maximum of 192 ppb.

Under normal barometric pressure (965 mbar) at a temperature of 20°C, water and CO₂ absorb NO, a process known as "quenching". This results in 2% lower NO determination than true levels. As the sensitivity of NO detection in the very low range varies by almost 10%, quenching-uncertainty is unlikely to be very important.

Volunteers and Patients

All volunteers breathed normally, in a comfortable position for 5–10 min (30–50 l), inhaling the purified air

from the first reservoir, with nose closed, *via* a T-air-valve and exhaling NO-containing air into a second flexible, empty reservoir. Reservoirs containing NO-free air were tested for NO immediately before use. At the end of the experiment, NO concentration in the second reservoir was analysed. To evaluate NO concentration in exhaled air of postoperative, hypertensive and dialysed patients, reservoirs with NO-free air were first analysed and then transported from the laboratory to the wards. Patients breathed normally for 5–10 min, inhaling the NO-free air from the first reservoir and exhaling their NO-containing air into a second empty reservoir as did the volunteers. To avoid a possible quenching of NO by CO₂, oxygen or water in the reservoirs containing volunteers' or patients' exhaled air, a water absorber was installed in the proximal expiratory port, and reservoirs were immediately transported back to the laboratory and analysed. In septic and intraoperative patients, purified air was not applicable due to mechanical ventilation. In these patients, routine inhaled air and exhaled air were collected in a comparable amount (30–50 l) at a minimal distance from the patient, transported to the laboratory and analysed immediately.

Eighty one healthy volunteers (33 females and 48 males) were allocated to four groups: nonsmoking females (n=21); smoking females (n=12); nonsmoking males (n=24); and smoking males (n=24). Volunteers with an asthmatic component, hyper- or hypotension, or infections within the previous two weeks were excluded. Smokers were obliged not to smoke within 2 h prior to NO measurements. Characteristics of volunteers are shown in table 1.

In addition to healthy volunteers, a total of 38 patients were recruited for the study. All patients or their legal representatives provided an oral informed consent. In 10 patients with documented hypertension, NO was analysed under medication. In 5 patients with renal failure (postpartum, membranous glomerular disease, chronic kidney rejection and immunoglobulin A (IgA)-nephritis), NO was analysed before and 1 h after the onset of haemodialysis. In 10 mechanically-ventilated, anaesthetized patients undergoing major surgery, NO was measured intraoperatively, 1 h after the induction of anaesthesia. In these

Table 1. – NO levels and characteristics of healthy volunteers

Group	Age yrs	Weight kg	Height cm	Pack yrs	HR b·min ⁻¹	MAP mmHg	SBP mmHg	DBP mmHg	NO ppb
Total (n=81)	30 ± 10	67 ± 11	174 ± 9		71 ± 8	95 ± 7	124 ± 10	80 ± 8	18 ± 8
NSF (n=21)	27 ± 10	59 ± 7	167 ± 4	0	72 ± 8	91 ± 10	119 ± 13	77 ± 9	21 ± 7
SF (n=12)	27 ± 5	58 ± 7	165 ± 9	7 ± 5	74 ± 6	92 ± 8	121 ± 8	78 ± 8	16 ± 6
NSM (n=24)	31 ± 9	72 ± 9	181 ± 6	0	69 ± 8	93 ± 7	125 ± 8	78 ± 7	19 ± 8
SM (n=24)	32 ± 11	73 ± 8	178 ± 6	11 ± 14	72 ± 8	100 ± 8	127 ± 9	86 ± 7	15 ± 6

Data are presented as mean $\pm$ SD. HR: heart rate; MAP: mean arterial pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure; ppb: parts per billion; NSF: nonsmoking females; SF: smoking females; NSM: nonsmoking males; SM: smoking males.

Table 2. – NO levels and characteristics of patients

Patient group	Age yrs	Weight kg	Height cm	HR b·min ⁻¹	MAP mmHg	SBP mmHg	DBP mmHg	NO ppb
Hypertension (n=10)	67 ±15	74 ±23	168 ±11	76 ±12	102 ±10	141 ±18	82 ±9	13.7 ±5.3
Renal failure b* (n=5)	40 ±18	64 ±17	169 ±8	77 ±13	112 ±13	156 ±14	98 ±17	20.2 ±6.8
Renal failure d* (n=5)		62 ±16		82 ±13	103 ±19	139 ±18	85 ±20	19.8 ±6.4
Intraop (n=10)	46 ±22	69 ±8	167 ±5	80 ±10	92 ±17	115 ±14	80 ±19	5.6 ±2.5
Postop (n=5)	52 ±17	70 ±8	172 ±12	86 ±16	91 ±10	125 ±13	75 ±10	10.3 ±3.5
Sepsis (n=3)	64 ±14	76 ±10	179 ±13	71 ±53	71 ±5	100 ±23	56 ±8	29.3 ±24

Data are presented as mean±SD. b*: before dialysis; d*: during dialysis; intraop: intraoperative patients; postop: postoperative patients; sepsis: septic patients in the intensive care unit. For further abbreviations see legend to table 1.

cases, the normal anaesthesia gas mixture (N₂O, enflurane and O₂) was applied as inhalatory air and NO concentrations were analysed. In 10 postoperative patients on days 3–5 and in 3 patients with a documented septic syndrome in the intensive care unit, NO was analysed. The body temperatures of septic patients were 37.9, 38.0 and 38.7°C, respectively. Leucocyte counts were 17.1, 13.3 and 7.8×10⁹·l⁻¹, respectively. All three patients were mechanically-ventilated with standard hospital air and oxygen. NO concentration of the inspired and expired air was assessed. Patients' characteristics are shown in table 2.

For statistical analyses the unpaired Student's t-test (two tail) was applied. Values of p≤0.05 were considered as criteria indicating statistical significance.

Results

Volunteers

NO concentration in exhaled air of all volunteers was 18±8 ppb, of nonsmoking females 21±7 ppb and of smoking females 16±6 ppb (p=0.029). Nonsmoking males

exhaled 19±8 ppb and smoking males 15±6 ppb (p=0.014). The difference between the smoking group (male and female) compared with the nonsmoking group (male and female) was statistically significant (p=0.004). Mean, systolic and diastolic blood pressures are reported in table 1. A statistically significant difference was found between diastolic blood pressure in smoking and non-smoking males (p=0.0008).

Patients

NO concentration in the exhaled air of patients with documented and treated hypertension was 13.7±5.3 ppb (table 2, fig. 1), significantly lower (p<0.05) than values found in healthy volunteers (18±8 ppb). Male patients with hypertension exhaled 10.2±2 ppb and females 17±5 ppb (p<0.05). Mean, systolic and diastolic blood pressure was 102±10, 141±18 and 82±9 mmHg, respectively.

NO concentration in the exhaled air of patients with renal failure was 20.2±6.8 ppb before and 19.8±6.4 ppb after 1 h of dialysis.

Fig. 1. – Mean NO levels in the exhaled air of the different volunteer and patient groups and relevant statistics. SP: smoking persons; NSP: non-smoking persons; TV: total volunteers (n=81); NSF: nonsmoking females, SF: smoking females; NSM: nonsmoking males; SM: smoking males; H: total hypertensive patients; HM: hypertensive males; HF: hypertensive females; RFb: renal failure patients before dialysis; RFd: renal failure patients during dialysis; IOP: intraoperative patients; POP: postoperative patients. Arrows indicate p≤0.05.

Intra- and postoperative NO exhalation was 5.6 ± 2.5 ppb and 10.3 ± 3.5 ppb. NO concentration of inhaled N_2O , enflurane and O_2 mixture was 4 ± 1 ppb.

In patients with sepsis syndrome, purified air was not applicable. Only three patients were involved. The first patient inhaled 7 ppb and exhaled 8 ppb NO; the second patient inhaled 43 ppb and exhaled 55 ppb; and the third patient inhaled 36 ppb and exhaled 25 ppb. Mean, systolic and diastolic blood pressures were 71 ± 5 , 100 ± 23 and 56 ± 8 mmHg, respectively.

Discussion

Endothelium-derived nitric oxide has been found to be a potent endogenous vasodilator with a major role in the regulation of peripheral tone in man [2–4]. Endogenous NO is present in the exhaled air of rabbits, guinea-pigs and humans [8], but its correlation with the serum NO levels is limited by the known contamination of ambient air with various amounts of NO. In the current study we have been able to produce an NO-free air supply, and to evaluate the levels of endogenous expired NO in healthy controls and individuals with various medical problems.

Nonsmoking healthy females exhaled relatively, but not statistically significantly, more NO than their male counterparts. Blood pressure levels were not significantly different. Cardiovascular risk of females of an age of less than 40 yrs is known to be reduced. An increased endogenous NO production in females may be an important factor indicating a relationship between the lower incidence of cardiovascular disease in woman and endogenous NO concentration.

Cigarette smoking seems to affect the levels of exhaled NO. We observed lower levels of expired NO in the smoking group (male and female) compared to the non-smoking group (male and female). Smoking males exhaled 4 ppb less NO than nonsmoking males, and smoking females exhaled 5 ppb less NO than nonsmoking females. In view of an increased NO concentration in the inspired air during smoking, lower NO concentration in the expired air in smokers could be explained either by decreased synthesis, a quantitative or qualitative increase of endogenous NO-synthetase-inhibitors, such as N^G -dimethylarginine or N^G -monomethyl-L-arginine [9], or by increased NO clearance.

In the group of smoking males, a significantly increased diastolic blood pressure was noted compared to non-smoking males. A large number of studies have confirmed an increased risk for hypertension in smokers, but the exact mechanism remains unclear. The above noted negative correlation between the amount of exhaled endogenous NO and smoking in males may be an important step in the understanding of the pathogenesis of hypertension in this group.

Patients with documented and treated hypertension were older, compared to the volunteer groups. There was a significant difference in mean and systolic blood pressure and exhaled NO concentration (5.3 ppb). Male patients with hypertension exhaled significantly less NO

than females (10 ± 2 versus 17 ± 5 ppb, respectively), and significantly less NO than all volunteer groups. Diastolic blood pressure was similar to volunteers, probably due to medication. A correlation between a decreased endogenous NO production and hypertension may be anticipated. An alternative hypothesis is that the antihypertensive medication influences NO production. A higher NO production due to medication or a more effective response to NO might lower blood pressure. Speculatively, endogenous NO production might be lower without medication. Nevertheless, the opposite might be possible. The medication administered could also lower endogenous NO production directly or *via* feedback mechanisms. Measurement of endogenously produced NO in untreated patients may be interesting.

In patients with renal failure, an insignificant but increased NO concentration was observed in exhaled air, without changes within an hour of dialysis. Blood pressure was found to be increased prior to dialysis. After one hour of dialysis, blood pressure decreased, probably due to an intended volume loss. These observations are entirely controversial, since increased concentrations of NO-synthetase-inhibitors were found in plasma and urine in patients with renal failure [9]. At the moment, the complexity of this observation needs clarification.

In intraoperative measurements, patients exhaled significantly less NO compared to volunteers. NO concentration of N_2O , enflurane and O_2 mixture (4 ± 1 ppb) was equivalent to NO-free air. A possible explanation for the low NO concentration may be an interaction with N_2O or enflurane. Another reason may be mechanical ventilation. In trauma patients, analogous low NO_2^-/NO_3^- levels were observed and remained low even in the presence of sepsis [10]. Considering a surgical procedure as a "trauma", our observations support these findings.

Postoperatively, a significant increase in NO was noted compared to intraoperative levels. As a significant difference to the volunteer group was still observed, the endogenous recovery of NO production may reflect convalescence.

In patients with sepsis syndrome, mean, systolic and diastolic blood pressures were significantly reduced and NO concentration significantly increased. However, the relevance of these results may be confounded by the varying concentrations in inhaled air. As purified air was not applicable in the inhalatory source, the statistically increased NO concentration of exhaled air may be not relevant. On the other hand, human serum levels of nitrite and nitrate ions significantly correlated with blood pressure [11]. High plasma levels of NO_2^-/NO_3^- were found in septic patients [10], and NO synthetase inhibitors were successfully utilized in combating hypotension in septic shock [12–14], indicating a role of NO in sepsis.

NO concentration of normal room-air was found to vary critically depending on the number of people and the position of windows in a room (6–192 ppb). Since 1981, the Office for Health and Environments of the City of Zurich performs NO concentration-measurements. As an interesting reference, mean NO concentration over

the year at one of the most heavily trafficked roads of Zurich in the area of the hospital was 45.5 ppb, reaching peaks of up to 170 ppb (Office for Health and Environments of the city of Zurich; personal communication). Against such background variation a standardized purified air for inhalation is mandatory for exact measurements.

The presence of NO in the exhaled air of anaesthetized rabbits, guinea-pigs and five humans has been demonstrated by chemiluminescence, diazotization and mass spectrometry in previous trials [8, 15]. Volunteers inhaled ambient air through the nose and exhaled 8 ± 0.8 ppb NO. The findings of this study substantiate NO exhalation. However, under well-controlled conditions we detected a notably higher NO concentration (18 ± 8 ppb) as well as a significant effect of smoking habit.

At the present time, various efforts are being made to use NO in ppm-concentrations in the treatment of adult respiratory distress syndrome, as well as in pulmonary hypertension. With regard to the calibrations-difference of a factor 1,000 the same NO-analyser is serviceable for NO inlet surveillance.

In view of our findings, efforts to perform NO blood gas analyses may be important. In septic shock, blood gas analyses may provide an important diagnostic tool in evaluating the state of sepsis and shock and determining exact indication and dosage of NO-synthetase-inhibitors [14]. In cardiovascular and hypertension research, effects of different therapies on NO concentrations may have several implications.

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