Reduced nitric oxide concentration in exhaled gas after exposure to hyperbaric hyperoxia.

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Taraldsøy T, Bolann BJ, Thorsen E. Reduced nitric oxide concentration in exhaled gas after exposure to hyperbaric hyperoxia. Undersea Hyperb Med 2007; 34(5):321-327. The objective of this study was to evaluate exhaled nitric oxide concentration (FENO) and exhaled breath condensate (EBC) pH and H₂O₂ as biochemical markers of pulmonary oxygen toxicity in association with hyperbaric oxygen (HBO₂) therapy. FENO, EBC pH and H₂O₂ were measured during the course of a 4 week HBO₂ treatment period, and the responses to a single HBO₂ exposure at the start and end of the treatment period were assessed. The HBO₂ exposure was at a pressure of 240 kPa for 90 min 5 days a week for 4 weeks. Eight patients undergoing HBO₂ therapy and eight control subjects participated in the study. There was a reduction in FENO immediately after HBO₂ exposure of 33.1 (SD=7.8) % on Day 1 and 40.7 (SD=8.9) % on Day 25. EBC pH was reduced after the first exposure only. Baseline FENO and EBC pH and H₂O₂ measured before the HBO₂ exposures did not change throughout the HBO₂ treatment period. A single HBO₂ exposure induces a significant transient decrease in FENO. Repeated exposures do not appear to induce inflammatory processes in the lung associated with an increase in FENO.

INTRODUCTION

Exposure to hyperoxia imposes an increased exogenous oxidant stress on the lung with long term exposure to hyperoxia. Some inflammatory processes in the lung are associated with an increased concentration of nitric oxide in exhaled gas. This is consistently demonstrated in asthma (4), and may be seen with exacerbations of chronic obstructive lung disease (5). Other biochemical markers of airway inflammatory processes may be found in condensate of exhaled gas, including a reduction in pH and an increase in hydrogen peroxide, products of lipid peroxidation and eicosanoids. The potential role of these exhaled biomarkers of pulmonary disease has been reviewed by Kharitonov and Barnes (6).

Pulmonary oxygen toxicity is well characterized pathophysiologically. There is a dose dependant reduction in vital capacity at
partial pressures of oxygen (PO₂) higher than 50 kPa (7). Reductions in maximal expiratory flow rates and transfer factor for carbon monoxide (TlCO) without changes in vital capacity have been demonstrated with continuous long term exposures to a PO₂ lower than 50 kPa in association with saturation diving operations (8,9). The hyperoxic exposure associated with hyperbaric oxygen (HBO₂) therapy amounts to a PO₂ of 200 - 240 kPa for 60-90 minutes daily over a treatment period of 20 – 30 days. Reductions in maximal expiratory flow rates and TlCO have been demonstrated in the course of HBO₂ therapy in subjects without lung disease and normal lung function at the start of treatment (10,11).

The objective of this study was to evaluate exhaled nitric oxide concentration (FENO) and exhaled breath condensate (EBC) pH and H₂O₂ as biochemical markers of pulmonary oxygen toxicity in association with HBO₂ therapy. It was hypothesized that if inflammation in the lung is induced as a result of this hyperoxic exposure, it would be accompanied by progressive changes in these variables and in maximal expiratory flow rates.

METHODS

Subjects
Eight patients undergoing hyperbaric oxygen therapy were consecutively selected for the study. They were receiving hyperbaric oxygen therapy for chronic radiation-induced injury. Patients with former irradiation of the head, neck and thorax as part of the treatment for the primary disease, any lung disease or current symptoms from upper or lower airways, known atopic disease and patients with radiologically abnormal findings in the lung parenchyma were excluded. Their mean age was 52.6 years (range 36 – 65) and there were 4 men. They were all never smokers. Two patients had undergone hyperbaric oxygen therapy two years or more before being included in this study.

Eight age-matched healthy subjects without any lung disease or current airway symptoms working in the hospital were selected as controls. Their mean age was 53.1 years (range 35 – 65) and there were 4 men. They were all never smokers.

The study was approved by the regional committee for medical research ethics and all subjects gave written informed consent.

Hyperbaric oxygen exposure
HBO₂ treatment was given once daily five days a week and included 20 HBO₂ exposures over a 26 day period. The patients were compressed with oxygen in a monoplace hyperbaric chamber to a pressure of 240 kPa within 10-15 min, and breathed oxygen at this pressure for 90 min in three cycles of 30 min interrupted by breathing compressed air from an oronasal mask for 5 min in between. Thereafter they were decompressed to normal ambient pressure in 7-10 min. The oxygen flow rate in the chambers was 100-200 L.min⁻¹, and was adjusted within this range for the regulation of the patients’ thermal comfort. Carbon dioxide concentration in the chambers was not monitored.

Study protocol
Measurements of exhaled nitric oxide concentration (FENO) and dynamic lung volumes, and collection of exhaled breath condensate were done within 60 min before and 15 - 30 min after hyperbaric oxygen exposure on the 1st and the 25th day of treatment (HBO₂ treatment day 19). The sequence of measurements was in this order every time. The measurements were done at least one hour after the last meal, and only drinking water was allowed until the post-exposure measurements were completed. The control subjects had the
same measurements done with similar time intervals as the HBO₂ exposed subjects on each of two days 24 – 28 days apart. They had the same restrictions on food and fluid intake as the patients. In addition to measurements on the 1st and 25th day the experimental subjects also had measurements before HBO₂ exposure on the 11th and the 18th day.

**Exhaled nitric oxide concentration**

FENO was measured intra-breath at an expiratory flow rate of 50 mL·s⁻¹ after inhalation from functional residual volume to total lung capacity using an online chemiluminescence analyser (Niox®, Aerocrine AB, Stockholm, Sweden). The mean value of three measurements not differing by more than 10% was used in the analyses.

**Spirometry**

Dynamic lung volumes were measured on a wedge spirometer (Vitalograph Ltd., Buckingham, England). Forced vital capacity (FVC) and forced expired volume in one second (FEV₁) were taken as the highest readings obtained from at least three satisfactory forced expiratory manoeuvres. Mean midexpiratory flow rate (FEF₂⁵-₇⁵%) was taken as the highest value from spirograms not differing by more than 5% from the highest FVC.

**Exhaled breath condensate**

Exhaled breath condensate collection was performed using the EcoScreen® (Erich Jaeger GmbH, Hoechberg, Germany). After 15 min 1.5 - 2.0 mL condensate was collected which was immediately flushed with medical quality compressed air for 2 min. The sample was then divided in 0.5 mL aliquots and pH was measured in one of these using an inoLab® pH meter (WTW GmbH, Weilheim, Germany). The others were immediately frozen at −80 °C for later analysis of H₂O₂. H₂O₂ was determined by reaction with horseradish peroxidase, reduction of the enzyme-substrate complex by para-hydroxyphenyl acetic acid and fluorescence detection of the reaction product (12). The limit of detection determined as 3 times the standard deviation of the blank was 40.2 nmol·L⁻¹. Analytical variation determined as pooled within-day coefficient of variation was 3.6 %. For each subject all samples were analysed on the same day, assuring a similar storage time of all samples from each subject.

**Statistics**

All results are given as mean (1 standard deviation) except for EBC H₂O₂ which was not normally distributed. Differences in biochemical and lung function variables during the 26 day course of HBO₂ treatment were tested by one-way repeated-measures analysis of variance. Changes in the biochemical and lung function variables after a single HBO₂ exposure on Day 1 and Day 25 were calculated as the percentage difference from the measurements before exposure on the same day. Differences from the baselines were tested using paired student’s t-test or Wilcoxon signed rank test. A p value <0.05 was considered to be significant.

**RESULTS**

The results of the measurements taken before HBO₂ exposures during the treatment period are given in Table 1 (see page 324 for Tables 1, 2, and Fig. 1). There were no changes in FVC and FEV₁, but a small reduction in FEF₂⁵-₇⁵% from 2.84 (1.02) L·s⁻¹ on Day 1 to 2.55 (0.88) L·s⁻¹ on Day 25 (p = 0.041). EBC pH, H₂O₂ and FENO measured before the HBO₂ exposures did not change during the treatment period.

The changes in the lung function variables immediately after a single HBO₂
Table 1. Dynamic lung volumes, exhaled nitric oxide concentration and exhaled breath condensate pH and H₂O₂ concentration in hyperbaric oxygen exposed subjects and control subjects. All measurements were done before hyperbaric oxygen exposure on the different days. Mean (1SD) or median (range).

<table>
<thead>
<tr>
<th></th>
<th>HBO₂ exposed subjects (n=8)</th>
<th>Control subjects (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 11</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>3.99 (0.79)</td>
<td>3.97 (0.79)</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>3.12 (0.67)</td>
<td>3.10 (0.64)</td>
</tr>
<tr>
<td>FEF₂₅-₇₅% (L·s⁻¹)</td>
<td>2.84 (1.02)</td>
<td>2.77 (1.02)</td>
</tr>
<tr>
<td>F₉NO (ppb)</td>
<td>20.5 (13.6)</td>
<td>21.1 (13.1)</td>
</tr>
<tr>
<td>EBC H₂O₂ (nmol·L⁻¹)</td>
<td>252 (73-618)</td>
<td>357 (47-1679)</td>
</tr>
<tr>
<td>EBC pH</td>
<td>7.20 (0.36)</td>
<td>6.79 (0.57)</td>
</tr>
</tbody>
</table>

* : Significantly different from Day 1, p<0.05. FVC: forced vital capacity. FEV₁: forced expired volume in one sec. FEF₂₅-₇₅%: mean maximal midexpiratory flow rate. F₉NO: nitric oxide concentration in exhaled gas. EBC: exhaled breath condensate.

Table 2. Changes (%) in dynamic lung volumes, exhaled nitric oxide concentration and exhaled breath condensate pH and H₂O₂ concentrations 15-30 min after a single hyperbaric oxygen exposure. Mean (1SD) or median (range).

<table>
<thead>
<tr>
<th></th>
<th>HBO₂ exposed subjects</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 25</td>
</tr>
<tr>
<td>FVC (% change)</td>
<td>-1.0  (3.0)</td>
<td>-0.8  (2.3)</td>
</tr>
<tr>
<td>FEV₁ (% change)</td>
<td>-0.8  (2.3)</td>
<td>1.9   (3.5)</td>
</tr>
<tr>
<td>FEF₂₅-₇₅% (% change)</td>
<td>2.6   (8.2)</td>
<td>-0.4  (11.5)</td>
</tr>
<tr>
<td>F₉NO (% change)</td>
<td>-33.1 (7.8)**</td>
<td>-40.7 (8.9)**</td>
</tr>
<tr>
<td>EBC H₂O₂ (% change)</td>
<td>80   (-80 - 332)</td>
<td>71   (-54 - 129)</td>
</tr>
<tr>
<td>EBC pH (% change)</td>
<td>-5.4  (4.8)*</td>
<td>-3.0  (6.2)</td>
</tr>
</tbody>
</table>

* : p<0.05. ** : p<0.001. FVC: forced vital capacity. FEV₁: forced expired volume in one sec. FEF₂₅-₇₅%: mean maximal midexpiratory flow rate. F₉NO: nitric oxide concentration in exhaled gas. EBC: exhaled breath condensate.

Fig. 1. Change in nitric oxide concentration (%) in exhaled gas after a single hyperbaric hyperoxic exposure compared with baseline nitric oxide concentration. The horizontal lines show mean changes in exposed subjects (filled circles) and control subjects (open circles).
exposure on Day 1 and Day 25 are given in Table 2. There was a reduction in $F_{e,NO}$ of 33.1 (7.8) % on Day 1 ($p<0.001$) and of 40.7 (8.9) % on Day 25 ($p<0.001$). The mean changes in $F_{e,NO}$ on Day 1 and Day 25 were not different. There was no relationship between the percent change in $F_{e,NO}$ and the absolute baseline value of $F_{e,NO}$, Figure 1. There was a small reduction in EBC pH on Day 1 ($p = 0.011$), but no change on Day 25. There were no significant changes in EBC $H_2O_2$, but there was a large variability in these results.

In the control subjects there were no significant differences in any of the lung function variables, Tables 1 and 2.

**DISCUSSION**

A substantial reduction in $F_{e,NO}$ 15-30 min after a single HBO$_2$ exposure was demonstrated. There was, however, no change in baseline $F_{e,NO}$ over the four week HBO$_2$ treatment period and the immediate response to a single HBO$_2$ exposure was the same at the start and end of the treatment period. This may indicate that the reduction in $F_{e,NO}$ is transient with complete recovery within 24 hours. A decrease in $F_{e,NO}$ immediately after a single HBO$_2$ exposure was also demonstrated by Puthucheary et al (13). The patients in that study had an increased baseline $F_{e,NO}$ compared to the control group, and follow up measurements during the course of HBO$_2$ treatment were not done. The hyperoxic exposure in this study was sufficient to reduce maximal expiratory flow rates by the end of the treatment period, as demonstrated in previous studies (10,11).

$F_{e,NO}$ is a balance between processes that produce and release NO into the airways and processes that scavenge NO. Several mechanisms with opposing effects on $F_{e,NO}$ may operate and compensate for each other with a net result of no change. The reduction in $F_{e,NO}$ immediately after HBO$_2$ exposure could be due to chemical reactions between reactive oxygen species and NO with formation of nitrates and nitrites (14). The reduction in EBC pH immediately after exposure on the first day of HBO$_2$ treatment may indicate the formation of nitric oxide metabolites.

The time interval between the end of the hyperoxic exposure and the measurement of $F_{e,NO}$ was 15 - 30 minutes. In the normal lung, washout is complete in about 5 min, and there were no changes in dynamic lung volumes or maximal expiratory flow rates immediately after single HBO$_2$ exposures. Alveolar PO$_2$ is expected to be normalised at the time of $F_{e,NO}$ measurements, unless changes in ventilatory control induced by the hyperoxic exposure resulted in changes in alveolar oxygen tension. It is a possibility that radical chain reactions initiated by reactive oxygen species and peroxynitrite are still propagating and scavenging nitric oxide after the end of HBO$_2$ exposure. Further studies of the time course of the changes in $F_{e,NO}$, alveolar O$_2$ and CO$_2$ tensions, and studies of nitric oxide metabolites in EBC could indicate whether such mechanisms are operative.

The finding of a reduced $F_{e,NO}$ after hyperbaric hypoxic exposure is in contrast with the findings after normobaric hypoxic and hypocoxic exposures where an increase in $F_{e,NO}$ with increasing PO$_2$ was demonstrated (15). However, in anesthetized ventilated subjects a reduction in $F_{e,NO}$ with increased PO$_2$ has been demonstrated, and there was an interrelation between oxygen and nitric oxide tensions having implications for gas exchange at the alveolar level (16). The alveoloarterial PO$_2$ difference increased with hyperoxia and there was a concomittant decrease in NO concentration. It is not known whether these mechanisms operate with exposure to hyperbaric hyperoxia.

Pulmonary oxygen toxicity has alveolar
and airway components (1,2). \( F_eNO \) was measured at one expiratory flow rate only of 50 mL \( \cdot \) s\(^{-1} \). At this flow rate the contribution of airway nitric oxide predominates. Partition of \( F_eNO \) into alveolar and airway contributions to the nitric oxide concentration can not be done unless \( F_eNO \) is measured at different expiratory flow rates (17). The implications for pulmonary blood flow and gas exchange might then be studied at times when the nitric oxide concentrations are maximally changed.

The \( F_eNO \) is significantly reduced in smokers compared with non-smokers (18). Cigarette smoke contains high amounts of oxidants in gas and particulate phase (19), and NO may be continuously scavenged by these oxidants resulting in a low \( F_eNO \). An upregulation of inducible nitric oxide synthase in the lung has been demonstrated in response to hyperoxic exposure but appears not to compensate for a reduction in exhaled \( F_eNO \) (20). With experimental exposure to ozone in concentrations known to induce airway inflammation no significant changes in \( F_eNO \) were demonstrated (21). The toxicity of ozone and hyperoxia is probably mediated by the same mechanisms, and inflammatory processes in the airways can not be excluded by the demonstration of no change in \( F_eNO \).

It has been shown that exposure to hyperoxia induces increased synthesis of anti-oxidant defense enzymes like superoxide dismutase and catalase (22,23), which could influence the EBC \( H_2O_2 \) concentration during the course of HBO\(_2\) treatment. These responses are more pronounced in pulmonary endothelial (22) compared with bronchial epithelial cells (23). EBC \( H_2O_2 \) is considered to be directly related to oxidant stress in the airways (24). Although not statistically significant the EBC \( H_2O_2 \) concentration tended to increase after the first HBO\(_2\) treatment, but less so after subsequent treatments. This is compatible with the hypothesis that oxidant stress can induce an increase in the endogenous antioxidant defense, so that subsequent oxidative stress is better tolerated (25). There was a large variability in the EBC \( H_2O_2 \) concentrations. The reasons for this are not known, but the variability in the HBO\(_2\) exposed group was considerably larger than in the control group indicating that the exposure could contribute to changes in EBC \( H_2O_2 \) concentration in some subjects.

The HBO\(_2\) exposed patients were compared with an external control group, and did not serve as their own controls by having hyperbaric exposure at a normal \( P_O2 \) of 21 kPa for the same time period. Exposure to 240 kPa for 90 min with a \( P_O2 \) of 21 kPa would be associated with an unacceptable risk of decompression sickness, being outside an acceptable diving table. An external control group not being exposed hyperoxia or hyperbaria was therefore established. Exposing healthy subjects to the same oxygen exposure over a 4 week period was also considered unacceptable due to acute and potential long term toxic effects of hyperoxia. Only patients with normal lung function and without clinical signs of ongoing inflammatory processes were selected to the study. It is possible however, that effects of previous radiation therapy, although not directed to the thoracic region, could blunt inflammatory responses or preconditioning of airway and alveolar epithelium. The number of subjects in the study was based on power analysis assuming that the standard deviation of the difference between repeated measurements of \( F_eNO \) is 15 % (26), and that an increase in \( F_eNO \) of 30 % or 4ppb or more is considered biologically relevant.

In conclusion, a single HBO\(_2\) exposure induces significant transient changes in \( F_eNO \) and EBC pH. Repeated exposures appear not to induce inflammatory processes in the airways that are detectable by changes in these biochemical markers, despite a small reduction in maximal expiratory flow rates.
ACKNOWLEDGMENTS

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REFERENCES