CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Supplementary oxygen in healthy subjects and those with COPD increases oxidative stress and airway inflammation

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Background: Hyperoxia increases oxidative stress through the generation of reactive oxygen species and may therefore enhance inflammation in the lungs. The aim of this study was to investigate whether short term supplementary oxygen (28%) increases oxidative stress and inflammation in the airways by measuring 8-isoprostane and interleukin 6 (IL-6) concentrations in exhaled breath condensate.
Methods: Twenty three healthy subjects (12 men, mean (SD) age 48 (7) years) and 23 patients with chronic obstructive pulmonary disease (COPD; 15 men, mean (SD) age 56 (5) years) were studied. 8-isoprostane and IL-6 concentrations were measured by immunoassay.
Results: Increased concentrations of 8-isoprostane and IL-6 were found in all subjects after breathing 28% oxygen for 1 hour. In healthy subjects the concentrations of 8-isoprostane and IL-6 were 10.9 (2.9) pg/ml and 4.9 (0.8) pg/ml, respectively, compared with baseline concentrations of 6.1 (1.3) pg/ml and 2.9 (0.6) pg/ml, and in patients with COPD the concentrations were 27.9 (3.1) pg/ml and 8.3 (1.2) pg/ml), respectively, compared with baseline concentrations of 18.9 (3.6) pg/ml and 6.3 (0.6) pg/ml. By contrast, breathing air through the same face mask for 1 hour had no significant effects on 8-isoprostane or IL-6 concentrations in normal subjects or those with COPD.
Conclusions: These findings suggest that short term supplementary oxygen may enhance oxidative stress and inflammation in the airways. Whether this happens with long term oxygen therapy needs to be determined.

There is accumulating evidence that oxidative stress may play an important pathophysiological role in chronic obstructive pulmonary disease (COPD) in which increased oxidative stress has been demonstrated, especially in severe disease and during exacerbations.[1 2] Supplementary oxygen therapy which is used in patients with severe COPD may further increase oxidative stress, theoretically resulting in enhancement of inflammation and worsening of the disease.[3] There is an increase in markers of oxidative stress in plasma and airways after hyperbaric oxygen therapy in humans, indicating that hyperoxia may worsen pulmonary disease toxicity.[4]

8-isoprostane has been used as quantitative index of oxidative stress in vivo.[5] Increased concentrations of exhaled 8-isoprostane have recently been demonstrated in several respiratory diseases associated with increased oxidative stress.[6] 8-isoprostane is also increased in bronchoalveolar lavage fluid of rats exposed to 90% oxygen for 48 hours.[7]

Interleukin 6 (IL-6) is a pro-inflammatory cytokine expressed in response to various inflammatory stimuli.[8 9] This cytokine has been used as a biomarker of inflammation and is increased in patients with COPD.[8] The validity of exhaled 8-isoprostane and IL-6 as markers of oxidative stress and inflammation has been confirmed in recent studies with exhaled breath condensate (EBC).[9]

Patients with severe COPD who develop cor pulmonale are commonly treated with long term oxygen therapy.[10] To investigate whether short term supplementary oxygen (28%) increases oxidative stress and inflammation in the airways we measured 8-isoprostane and IL-6 concentrations in EBC of healthy subjects and COPD patients after breathing room air and then after exposure to 28% oxygen given continuously through a face mask for 1 hour.

METHODS

Study population

Twenty three non-smoking healthy subjects and 23 COPD patients who were all confirmed ex-smokers (cigarette consumption 32 (5) pack-years) were studied (table 1). All subjects were recruited from the Respiratory Disease Institute, University of Bari and written informed consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee. The diagnosis of COPD was based on GOLD guidelines. Sixteen patients had mild disease (7 men, mean (SD) age 54 (4) years, forced expiratory volume in 1 second (FEV_1) 74 (5)% predicted) and seven were very severe (4 men, mean (SD) age 55 (2) years, FEV_1 27 (2)% predicted). The patients were clinically stable with no worsening of symptoms within the previous 8 weeks. The FEV_1/FVC ratio was unchanged over a period of at least 8 weeks before the study. All COPD patients were ex-smokers, had stopped smoking for at least 6 months, and were being treated with inhaled corticosteroids (fluticasone propionate 500–1000 μg daily or budesonide 400–800 μg daily) and inhaled β-adrenergic agonists. Those with very severe disease (Pa_{O_2} <8 kPa) were treated with domiciliary oxygen for 2–5 hours daily but none were receiving long term oxygen therapy. Healthy subjects were non-smokers who had no respiratory symptoms or respiratory tract infection for ⩾3 months before the study.

All subjects underwent a clinical examination and lung function 1 day before the collection of EBC. Patients with COPD treated with domiciliary oxygen suspended their

Abbreviations: COPD, chronic obstructive pulmonary disease; EBC, exhaled breath condensate; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL-6, interleukin 6

Table 1 Characteristics of study subjects

	COPD subjects receiving air (n = 5)	COPD subjects receiving oxygen (n = 18)	Healthy subjects receiving air (n = 5)	Healthy subjects receiving oxygen (n = 18)
Age (years)	53 (6)	55 (4)	47 (3)	46 (6)
Sex (M/F)	3/2	11/7	2/3	5/13
FEV_1 (% predicted)	60.9 (2.6)	54.1 (3.1)	101 (18)	103 (15)
FVC (% predicted)	72.1 (2.2)	75.2 (4.3)	119 (9)	120 (10)
Pa_{O_2} (kPa)	9.2 (0.4)	7.2 (0.4)	12.8 (0.4)	13.1 (0.3)
Pa_{CO_2} (kPa)	5.2 (0.2)	5.6 (0.2)	4.8 (0.3)	4.9 (0.2)

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity. Data are expressed as mean (SD).

oxygen treatment for 2 days before enrolment. Eighteen COPD patients (11 untreated and seven treated with domiciliary oxygen) and 18 healthy subjects breathed 28% oxygen continuously at tidal volume and normal breathing frequency through a face mask for 1 hour. The remaining subjects (five COPD and five healthy subjects) breathed ambient air through a face mask connected to a cylinder that was turned off for 1 hour (placebo). The selection of patients was random and the intervention blinded to the subject and the EBC analysis. Spirometric tests and EBC collection were performed before and after breathing oxygen or ambient air.

Pulmonary function testing

Pulmonary function tests were performed before the measurement of EBC using a dry spirometer (PK Morgan Ltd, Gillingham, Kent, UK).

Exhaled breath condensate

EBC was collected using a condenser (EcoScreen, Jaeger, Wurzburg, Germany) as previously described, with tidal breathing for 10 minutes.[9] Condensate (at least 1 ml) was collected on ice and stored at −70°C.

Assays

A specific enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) was used to measure 8-isoprostane and IL-6 concentrations in EBC. All values were measured in duplicate. The intra-assay and inter-assay variabilities were ⩽10%. The detection limits of the assays were 4 and 1.5 pg/ml, respectively.

Statistical analysis

Data are expressed as mean (SD) values. A Mann-Whitney test and a Wilcoxon matched paired test were used to compare the groups and correlations between variables were studied using Spearman's rank correlation test. A p value of <0.05 was considered statistically significant. 95% confidence intervals for any comparisons of non-parametric data between groups were used.

RESULTS

8-isoprostane

The baseline values of exhaled 8-isoprostane were significantly higher in patients with COPD than in normal subjects ($p<0.001$). Exhaled 8-isoprostane levels were higher in healthy subjects (n = 18) after 1 hour breathing 28% oxygen (10.9 (2.9) pg/ml) than in those breathing ambient air (6.1 (1.3) pg/ml, $p<0.0001$; fig 1A). A similar increase was observed in untreated COPD patients and in those treated with supplementary oxygen (27.9 (3.1) pg/ml) compared with the measurement after breathing ambient air (18.9 (3.6) pg/ml, $p<0.0001$; fig 1B). There was no significant difference between the baseline values and those after oxygen in patients with COPD and controls (p = 0.2).

The baseline concentrations of 8-isoprostane in patients with mild COPD and those with severe COPD on supplementary oxygen were 30.2 (1.1) and 27.6 (2.7) pg/ml, respectively. No statistically significant differences were found in the rise in 8-isoprostane concentrations after supplementary oxygen between COPD subjects previously treated with domiciliary oxygen and those untreated.

No statistically significant differences were observed in healthy controls (n = 5) or COPD patients (n = 5) after breathing ambient air through the same face mask for 1 hour (5.9 (2.2) and 19.1 (3.1) pg/ml *v* 6.3 (1.7) and 18.0 (2.7) pg/ml, p = 0.8 for both). No correlation was found between exhaled 8-isoprostane concentrations and FEV_1 or FVC. The reproducibility of exhaled 8-isoprostane measurements was assessed in 10 non-smoking normal subjects and 10 with COPD. For most measurements the differences between the two 8-isoprostane values lay within ±2SD (−0.14 (−0.32) pg/ml and −0.08 (1.09) pg/ml). The coefficient of variation for 8-isoprostane was 4.4% and 3.7%.

Interleukin-6

The baseline value of exhaled IL-6 was significantly greater in patients with COPD than in normal subjects ($p<0.001$). Exhaled IL-6 levels were higher in healthy subjects after 1 hour breathing oxygen at 28% (4.9 (0.8) pg/ml) compared with the measurement when breathing ambient air (2.9 (0.6) pg/ml, $p<0.0001$; fig 1C). A similar increase was seen in patients with COPD when breathing 28% oxygen (8.3 (1.2) pg/ml) compared with ambient air (6.3 (0.6) pg/ml, $p<0.0001$; fig 1D). There was no significant difference between the baseline values and those after breathing oxygen in individuals with COPD and controls (p = 0.2). The IL-6 baseline concentrations in the mild COPD group and the severe group on supplemental oxygen were 9.1 (0.7) and 7.6 (0.8) pg/ml, respectively.

No statistically significant differences were found in the rise in IL-6 concentrations after supplementary oxygen between COPD subjects treated with domiciliary oxygen and those who were untreated. No statistically significant differences were observed between healthy controls and COPD patients after 1 hour breathing ambient air (3.1 (0.7) and 6.1 (0.4) pg/ml *v* 2.7 (1.6) and 5.4 (0.7) pg/ml, p = 0.8 and p = 0.7) There was no correlation between the exhaled IL-6 concentration and either FEV_1 or FVC.

The reproducibility of exhaled IL-6 measurements was assessed in 10 non-smoking normal subjects and 10 with COPD. For most measurements the differences between the two IL-6 values were within ±2SD (−0.03 (−0.24) pg/ml and −0.04 (0.89) pg/ml). The coefficient of variation for IL-6 was 5.9% and 6.4%.

Lung function

No differences in FEV_1 and FVC were observed in healthy controls or in patients with COPD after 1 hour of breathing oxygen or ambient air.

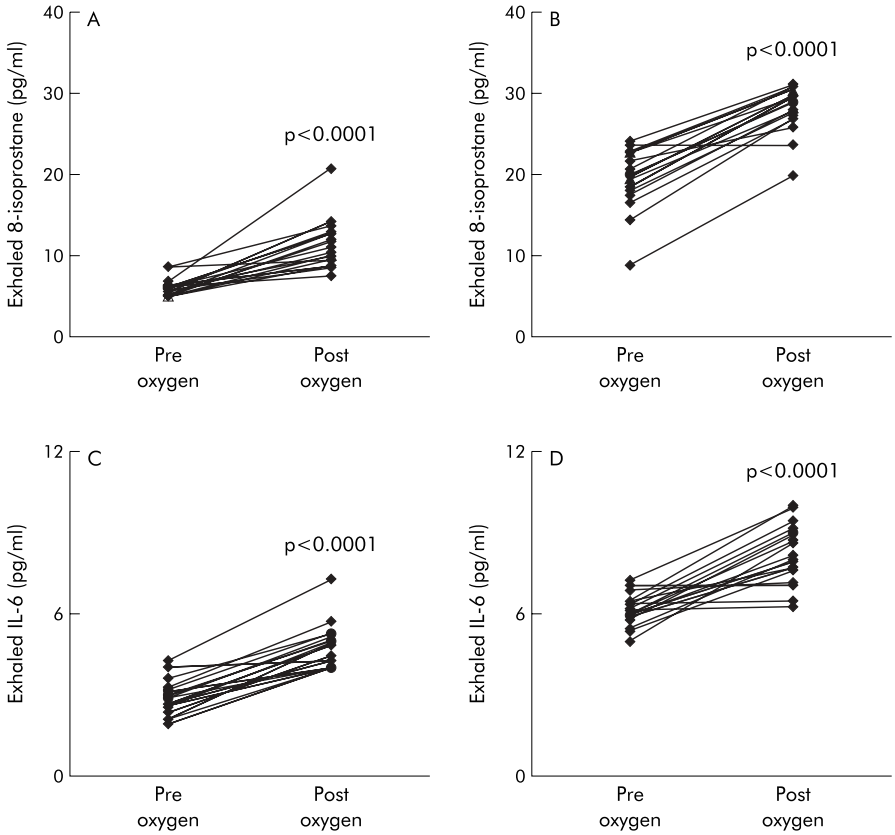

Figure 1 Exhaled 8-isoprostane and exhaled IL-6 concentrations before and after breathing 28% oxygen for 1 hour in (A, C) healthy subjects and (B, D) patients with COPD.

DISCUSSION

Our data show that the concentrations of both 8-isoprostane and of IL-6 are increased in the EBC of healthy subjects and COPD patients after short term oxygen breathing (28% oxygen by face mask for 1 hour). However, no differences were observed after breathing ambient air for the same period through the same face mask (placebo control). In this study we also confirmed previous data that show increased inflammation and oxidative stress in the airways of patients with COPD, with higher concentrations of 8-isoprostane and IL-6 in the EBC of these patients (especially in those with severe disease and using domiciliary oxygen therapy) than in healthy subjects.

Reactive oxygen species are produced endogenously by inflammatory cells or exogenously by inhalation of cigarette smoke, ozone, and nitrogen dioxide.[1] The increased oxidative burden is counteracted by efficient endogenous antioxidant defence mechanisms.[1 2] Excessive production of oxidants or reduced endogenous antioxidants results in increased oxidative stress which may further enhance pulmonary inflammation.[2] In this study we measured the effect of supplementary oxygen on the production of reactive oxygen species and on airway inflammation measured by exhaled 8-isosprostane and IL-6, respectively.

Several previous studies have reported that hyperoxia may increase oxidative stress in the lungs.[4 7] Recently, Phillips *et al* described an increase in breath methylated alkane concentration (BMAC) in normal healthy volunteers after breathing 28% oxygen for 20 minutes.[11] In agreement with these findings, we now report increased concentrations of 8-isoprostane in the EBC of healthy subjects exposed to 28% oxygen, which suggests that supplementary oxygen may increase oxidative stress in the lungs. To confirm that the exposure to 28% oxygen was itself responsible for this increase in oxidative stress, we administrated ambient air through a face mask in healthy subjects and those with COPD and did not find any increase in 8-isoprostane concentrations.

We also observed higher levels of 8-isoprostane in patients with more severe COPD treated with domiciliary oxygen than in those with mild untreated COPD. These results confirm the existence of a relationship between oxidative stress and disease severity[6] or suggest that supplementary oxygen, even when administrated for a short time, could contribute to increased oxidative stress.

We found a further increase in 8-isoprostane concentrations in EBC in patients with COPD (treated with domiciliary oxygen or untreated) exposed to 28% oxygen for 1 hour, indicating that even a modest increase in inspired oxygen concentration is sufficient to further increase oxidative stress, especially in COPD patients in whom baseline oxidative stress is already increased. This increased burden of oxidative stress may further increase the inflammatory response, as suggested in this study by the increased concentration of IL-6 which mirrors that of 8-isoprostane. This may be mediated through an increase in nuclear factor-κB, a transcription factor that is activated by oxidative stress and switches on the transcription of inflammatory genes such as IL-6.[2]

All the patients with COPD were treated with inhaled corticosteroids, but this is unlikely to have had an influence on our results since our previous studies have found no effect of even high doses of inhaled steroids on exhaled isoprostane levels in patients with asthma or in normal subjects exposed to ozone.[12] We believe that, although corticosteroids prolong

neutrophil survival, when administered by inhalation they may not influence the concentrations of mediators produced by neutrophils.[13] The fact that exhaled 8-isoprostane and IL-6 concentrations increase in patients with COPD on corticosteroids in a similar way to untreated normal subjects also suggests that corticosteroids are unlikely to have had an influence on the increase after supplementary oxygen.

Supplementary oxygen is clearly beneficial in patients with severe COPD and pulmonary hypertension with cor pulmonale. It has been shown to improve survival when given continuously and there are strict guidelines for its use.[14] However, the value of domiciliary oxygen in COPD patients with less severe hypoxia or with nocturnal desaturation has been questioned as there is no obvious clinical benefit.[15] Our results suggest that supplementary oxygen in the short term could have detrimental effects in patients with mild COPD by increasing the inflammatory response. This, supported by recent observations of Phillips *et al*, suggests that supplementary oxygen should be used cautiously in patients with COPD unless they have pulmonary hypertension as a result of severe hypoxia,[11] although hypoxia itself is sufficient to warrant this intervention.

However, it is possible that there is an adaptive response with long term administration with an increase in endogenous antioxidant mechanisms, so that the increase in oxidative stress and inflammation may not persist. Further studies of the effect of longer exposures to supplementary oxygen and at different oxygen concentrations are planned.

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REFERENCES

1 **Morcillo E**, Estrela J, Cortijo J. Oxidative stress and pulmonary inflammation: pharmacological intervention with antioxidants. *Pharmacol Res* 1999;**40**:394–404.
2 **Barnes PJ**. Chronic obstructive pulmonary disease. *N Engl J Med* 2000;**343**:269–80.
3 **Mantell LL**, Lee PJ. Signal transduction pathways in hyperoxia-induced lung cell death. *Mol Genet Metab* 2000;**71**:359–70.
4 **Bearden SE**, Cheuvront SN, Ring TA, *et al*. Oxidative stress during a 3.5-hour exposure to 120 kPa Pa_{O_2} in human divers. *Undersea Hyperb Med* 1999;**26**:159–64.
5 **Janssen LJ**. Isoprostanes: an overview and putative roles in pulmonary pathophysiology. *Am J Physiol Lung Cell Mol Physiol* 2001;**280**:L1067–82.
6 **Montuschi P**, Collins JV, Ciabattoni G, *et al*. Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am J Respir Crit Care Med* 2000;**162**:1175–7.
7 **Vacchiano CA**, Osborne GR, Tempel GE. 8-iso-$PGF_{2\alpha}$ production by alveolar macrophages exposed to hyperoxia. *Shock* 1998;**9**:266–73.
8 **Song W**, Zhao J, Li Z. Interleukin-6 in bronchoalveolar lavage fluid from patients with COPD. *Chin Med J* 2001;**114**:1140–2.
9 **Carpagnano GE**, Kharitonov SA, Resta O, *et al*. Increased 8-isoprostane and interleukin-6 in breath condensate of obstructive sleep apnoea patients. *Chest* 2002;**122**:1162–7.
10 **Tarpy SP**, Celli BR. Long-term oxygen therapy. *N Engl J Med* 1995;**333**:710–14.
11 **Phillips M**, Cataneo RN, Greenberg J, *et al*. Effect of oxygen on breath markers of oxidative stress. *Eur Respir J* 2003;**21**:48–51.
12 **Kharitonov SA**, Donnelly LE, Montuschi P, *et al*. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. *Thorax* 2002;**57**:889–96.
13 **Jatakanon A**, Uasuf C, Maziak W, *et al*. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 1999;**160**:1532–9.
14 **Nocturnal Oxygen Therapy Trial Group**. Continuous or nocturnal oxygen therapy in hypoxemic chronic obstructive lung disease: a clinical trial. *Ann Intern Med* 1980;**93**:391–8.
15 **Chaouat A**, Weitzenblum E, Kessler R, *et al*. A randomized trial of nocturnal oxygen therapy in chronic obstructive pulmonary disease patients. *Eur Respir J* 1999;**14**:1002–8.