must be carried out in a larger population to confirm our preliminary findings.

1 Bick RL. Coagulation abnormalities in malignancy: a review. *Semin Thromb Hemost* 1992;**18**:353–72.
2 Kwaan HC, Keer HN. Fibrinolysis and cancer. *Semin Thromb Hemost* 1990;**16**:230–5.
3 Gabazza EC, Taguchi O, Yamakami T, Machishi M, Ibata H, Suzuki S. Evaluating the prethrombotic state in lung cancer using molecular markers. *Chest* 1993;**103**:196–200.
4 Cox DR. Regression models and life tables. *J R Stat Soc* 1972;**187**:187–220.
5 Rickles FR, Edwards RL. Activation of blood coagulation in cancer: Trousseau's syndrome revisited. *Blood* 1984; **62**:14–31.
6 Constantini V, Zacharski LR. Fibrin and cancer. *Thromb Haemost* 1993;**69**:406–14.
7 Zacharski LR, Memoli VA, Constantini V, Wojtukiewicz MZ, Ornestein DL. Clotting factors in tumor tissue: implications for cancer therapy. *Blood Coagul Fibrinolysis* 1990;**1**:71–8.
8 Gasic GJ, Tuszynski GP, Gorelik E. Interaction of the hemostatic and immune systems in the metastatic spread of tumor cells. *Int Rev Exp Pathol* 1986;**29**:173–212.
9 Tryggvason KH, Salo T. Proteolytic degradation of extracellular matrix in tumor invasion. *Biochim Biophys Acta* 1987;**907**:191–217.
10 Wojtukiewicz MZ, Zacharski LR, Moritz TE, Edwards RL, Rickles FR. Prognostic significance of blood coagulation tests in carcinoma of lung and colon. *Blood Coagul Fibrinolysis* 1992;**3**:429–37.

Thorax 1997;**52**:565–568

Attenuation of oxidant/antioxidant imbalance during treatment of exacerbations of chronic obstructive pulmonary disease

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Abstract

***Background* – An oxidant/antioxidant imbalance is thought to occur in patients with chronic obstructive pulmonary disease (COPD). It has recently been shown that during exacerbations of COPD the antioxidant capacity and protein sulfhydryls of plasma are lower and the levels of products of lipid peroxidation are higher than in age matched healthy subjects. The aims of this study were to confirm these data and to measure the time course of these changes.**

***Methods* – The plasma Trolox equivalent antioxidant capacity (TEAC), protein sulfhydryls, and products of lipid peroxidation were measured throughout the course of treatment in 13 patients who presented with an acute exacerbation of COPD.**

***Results* – TEAC values (mmol/l) were low on admission (mean 0.67, 95% confidence interval (CI) 0.32 to 0.88; p<0.05) and had increased by discharge (0.98, 95% CI 0.88 to 1.2; p<0.05) but still remained lower than in healthy subjects (1.33, 95% CI 1.11 to 1.65). There was also restoration of plasma protein sulfhydryl levels (mmol/l) from admission (0.32, 95% CI 0.20 to 0.43) to discharge (0.49, 95% CI 0.42 to 0.62, p <0.001) to levels similar to those in healthy subjects (0.52, 95% CI 0.43 to 0.65). Products of lipid peroxidation, measured as thiobarbituric acid-malondialdehyde adducts (µmol/l), were significantly higher (2.08, 95% CI 1.8 to 2.5) than in control subjects (1.3, 95% CI 0.85 to 1.32; p<0.01) and returned to normal values by the time of discharge (1.2, 95% CI 0.88 to 1.29).**

***Conclusions* – These data confirm the presence of a profound oxidant/antioxidant imbalance in the blood of patients with acute exacerbations of COPD which returns towards normal values during the course of treatment.**

(*Thorax* 1997;**52**:565–568)

Keywords: oxidants, antioxidants, chronic obstructive pulmonary disease, lipid peroxides.

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Received 26 February 1997
Accepted for publication
27 February 1997

An oxidant/antioxidant imbalance is thought to play a part in the pathogenesis of chronic obstructive pulmonary disease (COPD).[1] Cigarette smoke and the release of reactive oxygen intermediates (ROI) from circulating neutrophils and airspace macrophages are major sources of oxidant stress in patients with COPD.[1]

There is, however, a paucity of data on the oxidant/antioxidant imbalance in patients with COPD, particularly during exacerbations. We have recently shown that the antioxidant capacity of plasma is lower during exacerbations of COPD than in age matched healthy subjects or patients with clinically stable COPD, suggesting increased oxidant stress.[2] This previous study was conducted in parallel groups of patients with either clinically stable or exacerbations of COPD. In the present study our aim was to assess the time course of the changes in markers of oxidant stress in plasma in individual patients during the course of an exacerbation of COPD.

Methods

STUDY POPULATION

Thirteen patients (six men) of mean (SD) age 69 (8) years who presented with an acute exacerbation of COPD were studied. The diagnosis of COPD was made by a respiratory

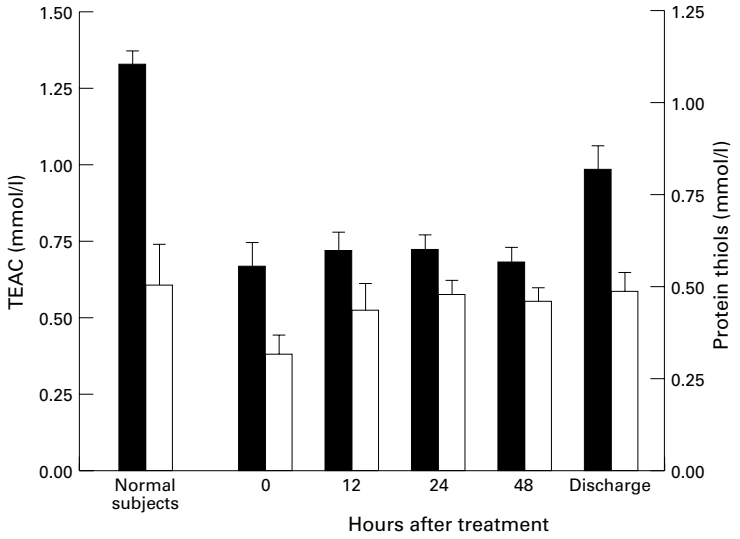

Figure 1 Time course of the changes in plasma Trolox equivalent antioxidant capacity (TEAC); solid columns) and protein thiols (open columns) in normal subjects and patients with exacerbations of COPD.

physician on the basis of current or ex-smoking and severe, largely irreversible, airways obstruction (<15% improvement in baseline FEV_1 in response to inhaled β_2 agonist). An exacerbation of COPD was defined as an increase above the patient's normal symptoms in two of the following: (1) breathlessness; (2) cough or sputum production; (3) sputum purulence, which was severe enough to warrant admission.

Six of the 13 patients had been started on oral corticosteroid therapy (20–40 mg prednisolone) during the week before admission. Prior to admission the other medications in these patients consisted of inhaled steroids and bronchodilator therapy in the form of β_2 agonists and/or ipratropium bromide. Treatment during the admission consisted of oxygen (1–3 l/min by nasal prongs), nebulised β_2 agonists, and ipratropium and continuation of corticosteroids in those who had received this treatment prior to admission. Six of the 13 patients were smokers and seven were ex-smokers who had quit smoking between six weeks and four years before their admission.

Eighteen healthy non-smokers (eight men) of mean (SD) age 59 (7) years randomly selected from the hospital staff who had no history of lung diseases were used as control subjects.

Blood samples were withdrawn from the patients within three hours of admission with an exacerbation of COPD and at 12, 24, and 48 hours thereafter. A further sample was taken 5–10 days later when the patient's condition was considered clinically stable enough for discharge. None of the patients smoked during their hospital admission. In the normal subjects blood samples were obtained on two occasions one week apart.

The study was approved by the local ethical committee and all patients gave written informed consent.

METHODOLOGY

For all of the assays described below 10 ml of venous blood was withdrawn into a lithium-heparin tube, centrifuged (250*g*), and the plasma removed and used immediately for the following assays.

Trolox equivalent antioxidant capacity (TEAC)

The plasma antioxidant capacity was measured by the method of Miller *et al*.[3] The Trolox equivalent antioxidant capacity (TEAC) was calculated by defining the concentration of Trolox in mmol/l having the equivalent antioxidant capacity to a 1.0 mmol/l sample of the plasma under investigation.

Products of lipid peroxidation

The concentration of products of lipid peroxidation in plasma as thiobarbituric acid (TBA)-malondialdehyde (MDA) adducts was measured by the method described by Yagi.[4] The final result was expressed as mol TBA-MDA adducts formed/litre of plasma.

Protein sulfhydryl and protein carbonyl assays

Protein thiols were measured using the method of Ellman[5] and protein carbonyls were assayed by the method described by Rodney and coworkers.[6]

We have already confirmed the sensitivity and reproducibility of these assays.[2]

STATISTICAL ANALYSIS

The data are expressed as mean and 95% confidence intervals (CI). Differences between mean values were assessed by a one way analysis of variance using Tukey's method.

Results

Arterial blood gas tensions on admission breathing oxygen (1–3 l/min by nasal prongs) were Pa_{O_2} 10.6 (0.9) kPa, Pa_{CO_2} 6.5 (0.5) kPa, H^+ ion 42.7 (1.6) nmol/l. None of the patients had clinical or radiological evidence of a pneumonia. Serum albumin and haemoglobin concentrations were 39.5 (1.3) g/l and 130.6 (15.3) g/l, respectively. The mean (SD) FEV_1 (% of predicted values) was 23 (7)% on admission, 26 (8)% 24 hours after admission, 31 (10)% at 48 hours, and 31 (6)% at discharge 5–10 days after admission.

The TEAC of plasma was lower in the patients at the time of their presentation with an acute exacerbation of COPD (0.67, 95% CI 0.32 to 0.88 mmol/l) than in normal age and sex matched healthy control subjects (1.33, 95% CI 1.11 to 1.65 mmol/l, $p<0.05$; fig 1). TEAC values were lower in those patients who were current smokers (mean 0.53 mmol/l, 95% CI 0.31 to 0.73; $n=7$) than in those who were ex-smokers (0.81 mmol/l, 95% CI 0.68 to 0.90, $n=6$; $p<0.05$). Patients who received treatment with steroids prior to admission had similar TEAC values (0.65 mmol/l, 95% CI 0.41 to 0.88; $n=6$) to those who had not received

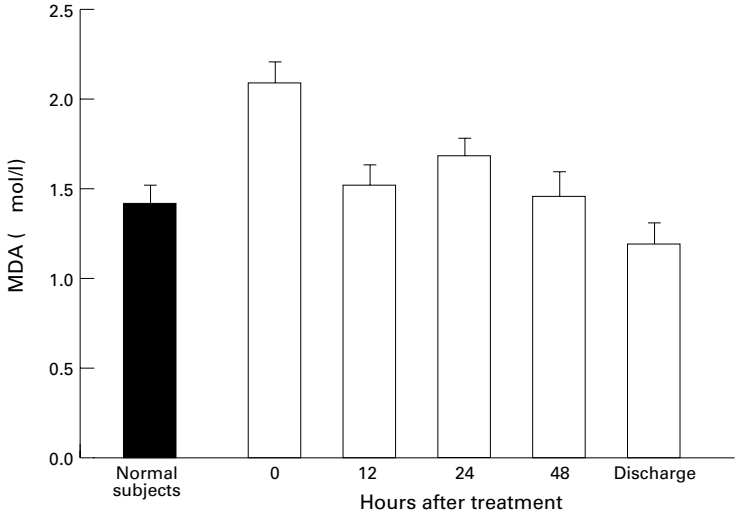

Figure 2 Time course of the changes in products of lipid peroxides, measured as TBA-MDA adducts, in patients with acute exacerbations of COPD.

this treatment (0.69 mmol/l, 95% CI 0.32 to 0.90; n = 7), and treatment with oxygen did not affect TEAC values (data not shown). TEAC of plasma remained low during the 48 hours following admission but had increased significantly by the time of discharge 5–10 days later. However, the levels were still lower than measurements made in normal subjects one week later ($p<0.05$). Multiple comparisons of TEAC measurements 12–48 hours after admission revealed no significant change (fig 1, $p = 1.0$).

Plasma protein thiols (mmol/l) were significantly lower in patients presenting with an acute exacerbation of COPD (0.32, 95% CI 0.20 to 0.42; $p<0.001$) than in normal subjects (0.52, 95% CI 0.43 to 0.65; fig 1). Previous or current history of smoking did not affect the levels of protein thiols (smokers 0.32 mmol/l, 95% CI 0.19 to 0.40, n = 7; ex-smokers 0.32, 95% CI 0.20 to 0.42; n = 6, $p = 1.0$). Protein thiols increased 12 hours after admission to 0.44 mmol/l (95% CI 0.36 to 0.56) and remained similar at the time of discharge (0.49, 95% CI 0.42 to 0.62; n = 18) to control subjects (0.50, 95% CI 0.42 to 0.64; n = 12, $p = 0.9$). The recovery of protein thiols was quicker than that of plasma TEAC. Multiple comparisons of protein thiol measurements between time points (12–48 hours) showed no significant difference ($p = 0.8$).

Plasma levels of products of lipid peroxidation, measured as TBA-MDA adducts (μmol/l), were higher in patients with COPD at the time of admission (2.08, 95% CI 1.8 to 2.5, n = 13; $p<0.01$) than in normal subjects (1.3, 95% CI 0.85 to 1.32; fig 2). TBA-MDA adducts decreased 12 hours after admission (1.50, 95% CI 0.9 to 1.46) and remained low until discharge, being similar to control values measured after an interval of one week (1.1, 95% CI 0.75 to 1.26; n = 12). Multiple comparisons between the different time points 12, 24, and 48 hours after treatment showed no difference in TBA-MDA adduct levels ($p = 0.9$).

Discussion

In a previous parallel group study we reported that the antioxidant capacity of plasma was reduced in smokers and in patients with exacerbations of COPD compared with age matched healthy subjects.[2] This was associated with increased superoxide anion generation by circulating neutrophils, which diminished by the time of discharge. In this study we confirm our earlier observations and provide further insights into the time course of the change in oxidant/antioxidant imbalance in acute exacerbations of COPD.

A persistently decreased antioxidant capacity was seen in the plasma of patients with an acute exacerbation of COPD for at least 48 hours following their admission, with a rise at the end of the exacerbation when they were considered to be clinically stable enough for discharge. However, in contrast to patients with clinically stable COPD who were studied at least six weeks after their last exacerbation,[2] TEAC values had not returned to normal levels.

A low TEAC in plasma suggests increased oxidant stress in the blood. As a result of oxidant stress, lipid peroxides are formed due to the peroxidation of unsaturated fatty acids present on cell membranes.[7] Our observation of increased levels of products of lipid peroxidation in plasma, together with a fall in antioxidant capacity in this and our previous study, strongly supports our contention that there is increased oxidative stress in patients with acute exacerbations of COPD. The increase in products of lipid peroxidation in plasma fell rapidly to normal values within 12 hours of admission and remained at these levels throughout the study. In contrast, plasma TEAC had not completely recovered to normal levels by the time of discharge.

The decrease in the TEAC was more pronounced in patients with a current smoking history than ex-smokers, which is in agreement with our earlier observations of a dramatic decrease in TEAC during smoking.[2] We also confirmed the results of our previous study that pretreatment with steroids did not affect TEAC values in patients with acute exacerbations of COPD.

In our previous study we found a correlation between plasma TEAC, the release of reactive oxygen intermediates (ROS) from circulating neutrophils, and protein sulfhydryls, suggesting a mechanism for the fall in plasma TEAC levels.[2] Several components of plasma contribute to plasma TEAC. Albumin, which has a sacrificial sulfhydryl group, is thought to account for two thirds of the plasma TEAC.[3] Many proteins such as ceruloplasmin, transferrin, and small antioxidant molecules such as non-protein thiols, vitamins C and E, and uric acid account for the remainder of the plasma TEAC. It has been reported that smoking is associated with a depletion of vitamin C and E levels in the blood.[8] The lower plasma TEAC in patients with acute exacerbations of COPD with a smoking history may therefore result from depletion of such antioxidant molecules. A dramatic fall in protein thiols is confirmed in this study in patients with acute exacerbation

of COPD. However, this depletion of protein thiols was restored during treatment of the exacerbation, beginning at 12 hours, and was back to normal levels by the time of discharge, while TEAC levels recovered significantly, but not to normal values, by the time of discharge. The increased oxidative stress measured as increased products of lipid peroxidation and decreased protein thiols normalised during the first 12 hours of treatment. However, a persistent depletion of other antioxidant molecules during exacerbations of COPD may account for the incomplete recovery of TEAC at or after 48 hours of treatment. Further studies are required to investigate this hypothesis.

The mechanism of the restoration of the plasma protein thiols during treatment of the exacerbation of COPD is unknown. Prednisolone stimulates the synthesis of thiols such as glutathione in the liver,[9] thus providing thiol groups to replenish protein thiols. However, in this relatively small group of patients there did not appear to be any relationship between the changes in plasma TEAC or protein thiols and any specific treatment, including corticosteroids. However, the possible role of steroids or other medications in inducing the recovery of protein thiols, and the antioxidant capacity of plasma during treatment of the exacerbation of COPD, requires further study in a larger group of patients. In addition, studies to monitor the levels of antioxidant molecules such as vitamins C and E, uric acid and beta-carotene during the course of acute exacerbations of COPD will help to elucidate the relationship between the fall in the antioxidant capacity of the plasma and the pathogenesis of exacerbations of COPD. Such studies may lead to the development of novel therapeutic interventions with antioxidant therapy in this condition.

These data add further support to the concept of an increase in oxidant stress in exacerbations of COPD, and point the way to further studies in this area.

This study was supported by the Chest, Heart and Stroke Association (Scotland) and the Norman Salvesen Emphysema Research Trust. The authors wish to thank Eileen Neal for typing the manuscript.

1 Rahman I, MacNee W. Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary disease. *Thorax* 1996;**51**:348–50.
2 Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD and smokers. *Am J Respir Crit Care Med* 1996;**154**:1055–66.
3 Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature babies. *Clin Sci* 1993;**84**:407–12.
4 Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* 1976;**15**:212–6.
5 Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;**82**:70–7.
6 Rodney LL, Galland D, Oliver CN, Amici A, Climent I, Lenz A, *et al.* Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990;**186**:464–78.
7 Pryor WA, Godber SS. Non-invasive measures of oxidative stress status in humans. *Free Radic Biol Med* 1991;**10**: 177–84.
8 Mazzetti A, Lapenna D, Pierdomenico SD, Calafiore AM, Costantini F, Riario-Sforza G, *et al.* Vitamin E, C and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. *Atherosclerosis* 1995;**112**:91–9.
9 Speck RF, Schrauz C, Lauterbrugh BH. Prednisolone stimulates hepatic glutathione synthesis in mice. *J Hepatol* 1993; **18**:62–7.