Oxidative Stress After Lung Resection Therapy*
A Pilot Study

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Study objectives: To investigate whether oxidative stress occurs following lobectomy and pneumonectomy and to evaluate whether markers of oxidative stress might be of value in the assessment of the diagnosis, course, and prognosis of postoperative complications.

Design: A prospective study.

Setting: A specialized thoracic surgical unit in a large referral hospital.

Patients: Twenty-eight patients with lung carcinoma undergoing thoracotomy.

Measurements: Exhaled H2O2 concentrations in breath condensate were measured by spectrophotometry, while malondialdehyde (MDA) levels in urine samples collected every 24 h were measured by reversed-phase, ion-pair high-performance liquid chromatography using ultraviolet detection.

Results: Our results show increased H2O2 and MDA levels in lobectomy patients compared with pneumonectomy patients. A strong correlation was found between the levels of H2O2 and MDA.

Conclusion: The present data support the hypothesis that oxidative stress may occur following pulmonary resection.

Key words: hydrogen peroxide; lobectomy; malondialdehyde; oxidative stress; pneumonectomy; predictive value of tests; pulmonary edema

Abbreviations: CI = confidence interval; MDA = malondialdehyde; PPE = postpneumonectomy pulmonary edema

Patients undergoing lung resection therapy may develop minor or major complications. To predict these postoperative complications, several tests (eg, measurement of FEV1, split function tests, and exercise tests) have been developed. Previous investigations have demonstrated a weak correlation between these measurements and the number or severity of postoperative complications.1–3 A retrospective analysis in our hospital among 197 patients who underwent pneumonectomy has not provided relevant clinical parameters accompanying the development of postpneumonectomy pulmonary edema (PPE).4 PPE is one of the most severe complications following thoracic surgery and has a high mortality rate.5 Waller et al6 suggested that PPE might have an etiology similar to ARDS. In addition, previous investigations have demonstrated that oxidative stress may be involved in the pathogenesis of ARDS.7–10 If similar pathophysiological mechanisms were involved in the development of PPE as compared to ARDS, the measurements of biomarkers of oxidative stress would be of interest in recognizing and observing these patients. Levels of H2O2 in expired breath condensate and of malondialdehyde (MDA) in urine are used as parameters for oxidative stress.11–13 H2O2 is a reactive oxygen species that reflects oxidative burden in the lungs. The presence of MDA in urine is classified as a systemic marker of lipid peroxidation.

The aim of the present study was to investigate whether oxidative stress may occur following pulmonary resection and to evaluate whether parameters of oxidative stress might be of value in the assessment of the diagnosis, course, and prognosis of postoperative complications, like PPE.

Materials and Methods

Patients and Control Subjects

Twenty-eight patients with lung carcinoma scheduled to undergo thoracotomy between January 1997 and April 1998 partic-
ipated in this study. The protocol was approved by the institutional review board. Informed consent was obtained from every patient. There were 10 male patients (age, 65 ± 8 years [mean ± SD]) undergoing pneumonectomy, and 14 male (age, 58 ± 16 years) and 4 female patients (age, 67 ± 9 years) undergoing lobectomy. All patients had smoked during their lives. Healthy volunteers (n = 24; 12 nonsmokers [6 women and 6 men] and 12 smokers [6 women and 6 men]) were enrolled for the measurement of reference values of H2O2.

Collection of Expired Breath Condensate and H2O2 Measurement

The patients’ samples of exhaled breath condensate were collected at 8:00 AM on the day of surgery, 30 min after surgery, and 1 day after surgery. The samples of healthy volunteers only were collected at 8:00 AM. Before collection, patients and control subjects rinsed their mouths with 0.2% chlorhexidine gluconate (Zeneca; Bidderkerk, The Netherlands) to exclude the effect of mouth flora. Subsequently, the participants breathed through a face mask with a two-way valve. The expired air was conducted through a connection with a “cold finger” collecting system that was connected to an 8-mL tube. In this way, approximately 1 to 4 mL of breath condensate was collected within 30 min with tidal breathing. After surgery, the collection system was connected to the expiratory port of the ventilator, since all patients were intubated. The samples were immediately frozen at −70°C. H2O2 measurements were performed within 1 week after sample collection, since preliminary data (results not shown) demonstrated that H2O2 concentrations had not changed during this period. The method described by Gallati and Pracht14 was applied. In this method, 100 μL 420 μmol/L 3,3’,5,5’-tetramethylbenzidine (Aldrich; Milwaukee, WI) were dissolved in 0.42 mol/L citrate buffer (pH, 3.8) (sodium citrate; Merck; Darmstadt, Germany; citric acid; Sigma; St. Louis, MO) and 10 μL 52.5 U/mL horseradish peroxidase (Sigma) were added to 200 μL condensate and to mixtures of 190 μL condensate and 10 μL 14,000 U/mL catalase (Sigma). Catalase was used to prove the specificity of the H2O2 measurement. The reaction proceeded for 30 min at room temperature. Subsequently, the mixture was acidified to pH 1 with 10 μL of 95 to 97% sulfuric acid (Baker; Deventer, The Netherlands). The reaction product was measured spectrophotometrically at 450 nm using an automated microplate reader (Titertek Twinreader type 380; Flow Laboratories Amstelstad B.V.; Zwanenburg, The Netherlands). The absorbance was directly proportional to the H2O2 concentration in the range of 0 to 10 μmol/L. All samples were measured in duplicate; mean values were used for subsequent analysis.

Collection of Urine and MDA Measurement

The patients’ samples of urine were collected on the day before, the day of, and the day after surgery. The urine samples collected every 24 h were collected in a container without any addition. MDA measurements were performed on the day of collection, since preliminary data (results not shown) demonstrated that MDA levels were changed during a 24-h storage time. Reversed-phase, ion-pair high-performance liquid chromatography using ultraviolet detection at 254 nm was used for the measurement.13 Aliquots of a urine specimen, filtered through a sterile 0.2-μm filter (Acrodisc; Gelman Sciences; Ann Arbor, MI), were analyzed using a high-performance liquid chromatography system (LKB-HPLC; Pharmacia Biotech AB; Uppsala, Sweden) and were separated on a 150 × 4.6-mm 3-μm packing column (Supelcosil LC-18HPLC; Supelco Inc; Bellefonte, PA) using a gradient elution. Absorbance was monitored, and data were collected with a databox (500 series, model 2600; Perkin-Elmer; Norwalk, CT) using computer software (Nelson Analytical Chromatography Software, revision 5.0, 1987, Perkin-Elmer). The following gradient was used for the chromatographic separation: 15 min in 100% of buffer A (10 mmol/L tetrabutylammoniumhydroxide [Sigma]/10 mmol/L KH2PO4 [Merck]; pH, 7.0); and 15 min in up to 100% of buffer B (2.8 mmol/L tetrabutylammoniumhydroxide /100 mmol/L KH2PO4; pH, 5.5), and the

![Figure 1. The 95% CI and the median of the responses of H2O2 and MDA, which is expressed as the ratio of the urinary creatinine values to lobectomy. pre = preoperative; post = postoperative.](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21942/ on 04/28/2017)
solution was held here for an additional 6 min. The initial conditions were restored after 9 min by washing with buffer A. A single assay was completed in 45 min. MDA concentrations were calculated by the measurement of peak-area ratios of the sample and the standard (1,1,3,3-tetraethoxypropane, 20 μmol/L; Sigma). The reproducibility of the method, expressed as the intrarun coefficient of variation, was 1.2% and the detection limit (blank value + 3 SDs) was 0.2 μmol/L. This method had a linearity up to 20 μmol/L. The MDA excretion is expressed as the ratio of the urinary creatinine.

Statistical Analysis

The measured values were nonnormally distributed. Thus, all statistical analyses followed the nonparametric approach. Consequently, all values are presented as median and the 95% confidence interval (CI). Comparisons within the lobectomy and pneumonectomy groups were made using the Wilcoxon signed rank test. The Mann-Whitney U test was used for comparisons between the groups. Statistical significance was assumed at p < 0.05. Spearman rank correlation tests were performed to detect a correlation between the concentration of exhaled H₂O₂ in breath condensate and the MDA excretion in urine samples collected every 24 h.

RESULTS

Patients who underwent lobectomy (Fig 1) exhibited a postoperative increase in the concentration of exhaled H₂O₂ (p < 0.001), whereas patients who underwent pneumonectomy exhibited no significant differences before and after the procedure (Fig 2). In the lobectomy patients, the postoperative H₂O₂ levels were significantly higher than in the pneumonectomy patients (p < 0.05). One day after thoracotomy, the concentrations of expired H₂O₂ returned to baseline values.

MDA excretion increased considerably in patients who underwent lobectomy (p < 0.001), while it slightly increased in those who underwent pneumonectomy (p = 0.004). The postoperative MDA levels showed highly significant differences between the lobectomy and pneumonectomy groups (p < 0.0005). MDA levels returned to baseline values 1 day after surgery in patients who underwent thoracotomy (Figs 1 and 2).

A strong correlation (r = 0.79) was found between the levels of H₂O₂ and MDA (n = 18) in patients who underwent thoracotomy (Fig 3). In 20 cases the concentration of H₂O₂ in breath condensates was below the detection limit of 0.14 μmol/L.

DISCUSSION

Oxidative stress, an imbalance between oxidants and antioxidants, is suggested as a possible pathophysiologic feature in many pulmonary diseases like COPD and ARDS. In this pilot study, we explored the hypothesis that oxidative stress may occur following pulmonary resection. Toward this view, we compared the level of oxidative stress in patients undergoing lobectomy or pneumonectomy.

Before discussing the implications of our findings, we should address some possible limitations of our...
First, it was not possible to obtain all data points in all patients. Among particular missing values were those belonging to the time point 1 day after surgery. In our statistical analysis, we corrected for these missing data because fewer values lead to a broadening of the 95% CIs. Second, we cannot exclude the possibility that mechanical ventilation affects H$_2$O$_2$ and MDA levels due to intubation. However, all patients were intubated and receiving mechanical ventilation for the same time period. Therefore, any possible effect of intubation on H$_2$O$_2$ and MDA levels may be assumed to be constant in both groups. Consequently, the observed differences are valid. Besides, intubation is unavoidable in these patients.

Our results show that in lobectomy patients the concentrations of exhaled H$_2$O$_2$ and MDA excretion were higher after surgery than in the pneumonectomy patients. This might be explained by the fact that in patients undergoing lobectomy the lobes have to be separated by the surgeon, which means much more manipulation than in a patient undergoing pneumonectomy. Therefore, lobectomy seems to be more stressful. Besides, the remaining lobe seems more prone to oxidative stress than an empty cavity after pneumonectomy.

Recently, Williams et al$^{18}$ found changes in several markers of oxidative stress (protein thiol, protein carbonyl, and myeloperoxidase levels) following lung resection. They expected that the lobectomy patient group would be subjected to a greater degree of oxidative stress than the pneumonectomy group, but they were not able to conclude this from their results. A possible explanation might be that the parameters, which were measured in plasma, do not reflect the degree of oxidative stress in the lung. In this study, we used direct measurement of oxidative stress in the lung by exhaled H$_2$O$_2$. This method should be able to distinguish between lobectomy and pneumonectomy, assuming the higher stress in lobectomy.

Only one patient developed PPE in our pilot study. This could be expected because the frequency of PPE following pneumonectomy is 4%. No other postoperative complications were encountered. Nine pneumonectomy patients showed the same downward trend after surgery, while the PPE patient displayed significantly elevated levels of H$_2$O$_2$ and MDA in comparison with the other pneumonectomy patients. Because only one patient in our study developed postoperative complications, it is possible only to indicate a trend.

The fact that the increases in levels of H$_2$O$_2$ and MDA were similar suggests that a relationship between these two parameters for oxidative stress might exist. Indeed, we found a significant correlation between the levels of H$_2$O$_2$ and MDA in thoracotomy patients. This correlation between two different classified and measured parameters in different patients strengthens the hypothesis that oxidative stress may occur following pulmonary resection.

In conclusion, the results of the present study support the hypothesis that oxidative stress may occur following lung resection. Lobectomy patients
showed higher \( \text{H}_2\text{O}_2 \) and MDA levels than pneumonectomy patients. In one PPE patient, both \( \text{H}_2\text{O}_2 \) and MDA levels increased steadily in comparison with the other pneumonectomy patients. Large-scale prospective studies are indicated to explore the potential predictive value of these types of markers.

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REFERENCES