One lung ventilation increases oxidative damage to proteins

Wentylacja rozdzielna nasila oksydacyjne uszkodzenie białek

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Abstract

Introduction: Hypoxia and hypoperfusion of the non-ventilated lung results in increased levels of reactive oxygen species, which may take part in the pathomechanism of complications after lung resection. The aim of the study was to evaluate the risk factors of increased oxidative stress during pulmonary resection. The influence of oxidative stress on the postoperative complications was recorded.

Material and methods: Forty patients entered this prospective study. Patients were distributed into one of the two arms of the trial undergoing one or two lung ventilation during the resection. Blood was collected at the determined time points. The levels of carbonyl and sulfhydryl remnants were assessed.

Results: Multivariate analyses showed that one lung ventilation was an independent risk factor of increased oxidative stress as measured by the decreased level of sulfhydryl remnants. The complication rates in both arms did not significantly differ.

Conclusions: One lung ventilation is an independent risk factor for increased oxidative stress. This study did not show an influence of oxidative stress on the complication rate after lung resection.

Key words: pulmonary resection, lung cancer, one lung ventilation, oxidative stress.

Streszczenie

Wstęp: Rozdzielenie wentylacji płuc na czas resekcji miąższu może być powodem powstania urazu o charakterze niedokrwienia – reperfuzji w pozostałym, niewentylowanym w czasie zabiegu płacie lub płatach płuca. Celem badania była ocena wpływu rozdzielenia wentylacji płuc w trakcie resekcji miąższu płuca na nasilenie stresu oksydacyjnego w okresie okołooperacyjnym, przebieg kliniczny oraz występowanie powikłań w okresie okołooperacyjnym.

Materiał i metody: Do badania prospektywnie włączono 40 pacjentów, kwalifikując ich do dwóch ramion badania. Pacjenci w trakcie resekcji miąższu płuca poddawani byli wentylacji rozdzielnej lub wentylacji obu płuc. Oznaczano stężenie białka, grup karbonylowych i sulfhydrylowych będących wynikiem oksydacyjnego uszkodzenia białek.

Wyniki: W analizie wieloczynnikowej jedynym niezależnym czynnikiem ryzyka wystąpienia nasilonego stresu oksydacyjnego wyrażonego spadkiem stężenia grup sulfhydrylowych było zastosowanie rurki dwuświatłowej i rozdzielna wentylacja płuc w trakcie zabiegu. Różnice w częstości występowania powikłań nie różniły się w badanych grupach.

Wnioski: Rozdzielenie wentylacji płuc w trakcie zabiegu resekcji miąższu płuca jest niezależnym czynnikiem ryzyka nasilenia stresu oksydacyjnego. Rozdzielenie wentylacji w trakcie resekcji miąższu płuca nie ma wpływu na częstość występowania powikłań pooperacyjnych ani na pooperacyjny przebieg kliniczny.

Słowa kluczowe: resekcja miąższu płuca, rak płuca, wentylacja rozdzielna, stres oksydacyjny.

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Introduction

Complication rates after major pulmonary resection are high in patients with significant concomitant diseases. The increasing number of challenging patients subjected to surgery, despite recent contraindications such as severe chronic obstructive pulmonary disease (COPD) or renal failure after neoadjuvant chemotherapy, convinces clinicians to reassess risk factors influencing the postoperative clinical course. It is not possible to define a single key factor in the pathology of complications following pulmonary resection. Studies investigating the whole spectrum of potential factors may highlight the influence that postoperative clinical course can have on the prevention of complications, decreasing mortality and shortening of the postoperative stay [1].

One lung ventilation (OLV) has been routinely used in video-assisted surgery but is not mandatory in open lung surgery. OLV causes over 60% decrease in blood flow through the pulmonary circulation of the non-ventilated lung. Diminished distribution of blood is caused by hypoxic pulmonary vasoconstriction [2] and gravitational hyperemia of the ventilated lung. Temporary ischemia that occurs during OLV, followed by reperfusion after restoring ventilation, in combination with iatrogenic hyperinflation of the ventilated lung, is a trigger that leads to overproduction of reactive oxygen species (ROS). The mechanisms resulting in the overproduction of ROS are the most thoroughly analyzed cellular effects of ischemia-reperfusion injury (IRI) [3, 4].

ROS have been suggested to have an influence on the rate of postoperative complications in general thoracic surgery. Lipids modified by ROS take part in the pathomechanism of atrial fibrillation after pulmonary resections [5]. ROS levels in serum are increased in different pathological situations such as acute lung injury (ALI), adult respiratory distress syndrome (ARDS), exacerbation of COPD and asthma or pneumonia.

The changes in the concentration of the molecules containing oxygen with unpaired electrons on valence orbitals are difficult to assess due to their highly reactive character. Indirectly, *in vivo* models have shown that general ROS production may be estimated on the basis of changes in the concentrations of the products created due to oxidative damage to proteins. The increase in the concentration of carbonyl groups in the serum is the result of oxidization of an ammonium group by hydroxyl radicals that oxidize the protein molecule leading to the creation of protein dimers. Excessive oxidation decreases the concentration of sulfhydryl groups associated with cysteine and methionine residues, resulting in their covalent binding.

Material and methods

Patient inclusion and exclusion criteria for the study are listed in Table I. The study was approved by the Institutional Review Board, Medical University of Gdansk. After written informed consent, patients were enrolled in one of two arms of the trial. Patients in the study arm – group I – after double-lumen endobronchial tube intubation, underwent OLV during the surgery. In the control arm – group II – patients underwent ventilation of both lungs.

Anesthesia was induced with thiopental (5 mg/kg) or propofol (2 mg/kg) or ethomidate (0.3 mg/kg), fentanyl (2-3 µg/kg) and vecuronium (0.1 mg/kg) or rocuronium (0.6 mg/kg) or cis-atracurium (0.15 mg/kg). Patients were ventilated with N_2O and O_2 in volumetric ratio 2 : 1 or 1 : 1 and 0.5-3% sevoflurane or 2-6% desflurane or 0.5% isoflurane. The tidal volume was 4-6 mL/kg, respiratory rate 10-15 per minute, fraction of inspired O_2 0.3-0.5 (in hypoxia up to 1), inspiratory expiratory rate 1 : 2, peak airway pressure up to 35 cm H_2O.

Blood samples (4 mL) were collected the day before surgery (T1), after the induction of anesthesia before turning to the lateral decubitus position (T2) and after resection of the lung (clamping of relevant pulmonary vein) (T3). The last sample was collected 24 hours after surgery (T4). Blood was collected in tubes containing ethylenediaminetetraacetic acid. Platelet rich plasma was separated (900 rpm, 10 minutes), and stored at –70°C until laboratory analysis was performed (within 6 weeks).

The level of plasma protein was assessed using a biuret reaction at the absorbance of 540 nm. The sulfhydryl remnant concentration was determined by reaction of plasma with KCl and tris(hydroxymethyl)aminomethane after sequential adding of sodium sulfo-dodecylamine, $NaPO_4$ at pH 8.0 and 2,2'-nitro-5,5'-dithiobenzoic acid. The absorbance was read at 412 nm. The carbonyl remnant concentration was estimated after incubation of plasma with (2,4-dinitrophenyl)hydrazine and after adding trichloroacetic acid. The so-

Tab. I. Study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria	Exclusion criteria during the study period
• resectable lung tumor • FEV_1 > 1.5 l • NYHA < II • performance status PS = 0-1 • ASA I-II • BMI > 21 BMI < 30 kg/m^2 • signing of informed consent	• contraindication for double lumen intubation • radiological features of active tuberculosis • long-lasting treatment with medication affecting lung parenchyma (i.e. amiodarone) • inflammatory process in lung parenchyma or pleural cavity • weight loss of more than 10% in 6 months • diseases impairing postoperative rehabilitation	• massive intraoperative hemorrhage • intraoperative incidental release of significant amounts of blood, pus or lung tissue into the bronchial tree

FEV_1 – forced expiratory volume in 1 second; NYHA – New York Heart Association Heart Failure Scale; ASA – American Society of Anesthesiologists Scale; BMI – body mass index.

lution was rinsed with ethanol and the guanidine was added to the precipitate. The absorbance was read at 360 nm.

Evaluation of risk factors for increased oxidative stress required data categorization. In accordance with Wiliams et al. [6], a 10% decrease of sulfhydryl concentration or 5% increase of carbonyl concentration was considered to be significant. The preoperative concentration (T1) was compared with post-induction (T2), intraoperative (T3) and postoperative (T4) concentration.

Patient group characteristics were presented as mean (standard deviation, SD) or median (range) depending on the type of data and distribution. Normal distributions were verified with the Shapiro-Wilk W test. The data were compared using Student's t test or the Mann-Whitney U test. Repeated measurements were analyzed using a two-way ANOVA test for repeated measurements, followed by analysis of significant differences using the post hoc method (Tukey's honestly significant difference test) when appropriate. Categorical data were presented as percentages with 95 per cent confidence intervals and compared using a chi square test, with Yates' correction when necessary. The level of significance of $p < 0.05$ was adopted. STATISTICA v. 7.1 PL (StatSoft, Tulsa, USA) was used for statistical analysis.

Results

Between September 2006 and June 2008, 40 patients were enrolled in one of two study arms. None of the patients was excluded during the study. Patient characteristics in the study groups are listed in Table II. The patients in group I were intubated with a double lumen endobronchial tube. OLV was initiated a few minutes before thoracotomy in order to provide sufficient atelectasis before opening the pleural cavity. The mean time of OLV was 75 minutes (SD = 38; 95% confidence interval CI 57-93). The anesthetics used for induction and conduction of anesthesia as well as the intraoperative fluid load did not differ significantly between the study groups.

All patients were operated on in the lateral decubitus position through postero-lateral incision. The type of surgeries performed are listed in Table III. The time between the intubation and clamping of the relevant pulmonary vein (that functionally ended up the resection) did not differ between study groups: 47 minutes in group I (SD = 22, 95%CI 37-58) and 52 minutes in group II (SD = 22, 95%CI 41-62) ($p = 0.54$). The mean time of surgery was 124 minutes (SD = 30, 95%CI 110-138) in group I, and 126 minutes in group II (SD = 44, 95%CI 106-147) ($p = 0.86$). Despite two pneumonectomies performed in group I in contrast to none being performed in group II, univariate analysis did not reveal a significant influence of type of operation on increased oxidative stress (decreased sulfhydryl concentration) ($p = 0.637$ for pneumonectomy, $p = 0.938$ for lobectomy and $p = 0.742$ for wedge resection). None of the medications used for anesthesia proved to have an independent influence on exaggerated oxidative stress.

Non-small cell lung cancer (NSCLC) was the most common histology of resected tumors in group I (15 patients; 75%) and in group II (13 patients; 65%) ($p = 0.49$), whilst tuberculomas were the most common non-malignant lesions resected. Active tuberculosis was excluded preoperatively in all patients.

There were no significant differences in the changes of either sulfhydryl ($p = 0.077$) (Fig. 1) or carbonyl groups ($p = 0.277$) (Fig. 2) between study groups at the defined time points. In order to obtain a normal distribution, data in Figure 2 are presented as a negative logarithm of the sulfhydryl concentration. A significant intraoperative decrease (T1 vs. T3) in plasma protein concentration was found only in the OLV group ($p = 0.01$).

Multivariate analysis showed that one lung ventilation was the only independent risk factor of increased oxidative stress, as expressed by a 10% decrease in the level of sulfhydryl remnants ($p = 0.032$; odds ratio OR = 8.257; 95%CI OR: 1.216-56.054).

There was no significant impact on oxidative stress of the following tumor features: type of NSCLC, pathological stage of NSCLC.

The postoperative complications are listed in Table IV. The most common complication was atrial fibrillation, which occurred more frequently in the OLV group. However, the difference was not significant. No significant influence of ventilation type on the complication rate was observed.

Discussion

Recently published studies revealed excessive production of ROS during pulmonary resections [5, 7]. Our results, following multivariate analysis, proved that OLV is a risk factor of exaggerated intraoperative oxidative stress, as expressed by a 10% decrease of sulfhydryl remnants con-

Tab. II. Patients' characteristics in study groups

Feature	Group I 20 patients	Group II 20 patients	*p* value
age (years)	62	61	0.646
gender male : female	7 : 13	15 : 5	0.01
FEV1 [l]	2.28	2.38	0.7
FEV1% [%]	84.06	78.82	0.47
FVC [l]	3.18	3.60	0.19
FVC% [%]	92.66	94.71	0.77
package years	28	34	0.44
active smokers	13 (65%)	14 (70%)	0.73

Mean values (age, FEV1), median values – remaining features. FEV1 – forced expiratory volume in 1 second; FVC – forced vital capacity; COPD – chronic obstructive pulmonary disease.

Tab. III. Types of surgery in study groups

Type of surgery	Group I 20 patients	Group II 20 patients	*p* value
wedge resections	4 (20%)	6 (30%)	0.488
lobectomy	14 (70%)	14 (70%)	
pneumonectomy	2 (10%)	0	

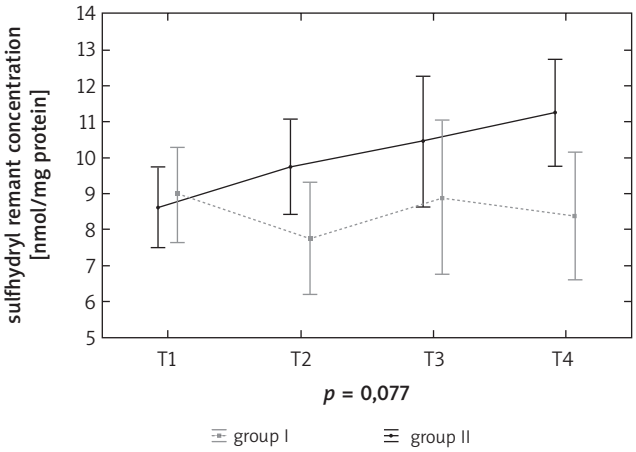

Fig. 1. Sulfhydryl concentration at time points (T) in study groups (mean and 95%CI)

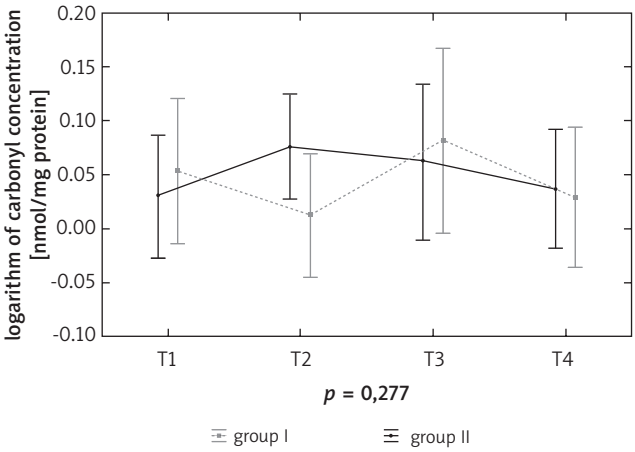

Fig. 2. Negative logarithm of plasma carbonyl concentration at time points (T) in study groups (-logarithm of carbonyl concentration and 95%CI)

Tab. IV. Complication rate in study groups

	Group I 20 patients	Group II 20 patients	*p* value
postoperative complications			
total number of complications	7 (35%)	3 (15%)	0.136
atrial fibrillation	4 (20%)	1 (5%)	0.170
atelectasis requiring bronchofiberoscopy	1 (5%)	1 (5%)	0.500
pleural empyema	1 (5%)	0	0.500
rethoracotomy due to hemorrhage	1 (5%)	0	0.500
hemorrhage from digestive tract	1 (5%)	0	0.500
exacerbation of chronic renal failure	0	1 (5%)	0.500
length of postoperative course (days)			
	6 (3-13)	6 (3-16)	0.725

centration during a pulmonary resection, which is consistent with previous studies [6]. Proteins are relatively resistant to oxidative stress. We did not observe an increase in carbonyl concentration, in contradiction to other studies, where a 5% increase was shown [6]. In our study OLV has been shown to be a risk factor of oxidative damage to proteins. Significant changes in the concentration of lipid peroxidation derivatives in the plasma during OLV depend on the length of atelectasis [5, 7]. In our study, due to the non-specific reaction of thiobarbituric acid with MDA, we evaluated more stable molecules. In future studies, different, strictly specific markers such as 4-hydroxynonenal [8] or isoprostanes should be implemented as measures of lipid peroxidation.

OLV during pulmonary resection does not result in direct IRI such as during organ transplantation. Nevertheless, a significant decrease in blood flow through the non-ventilated lung, together with hypoxia, could be a trigger of IRI. Hypothetical IRI occurring during OLV results in overproduction of ROS – a mechanism that correlates the length of the ischemia period with the degree of oxidative damage to lipids [4, 5, 7, 9]. Studies that compare the oxidative balance during different types of pulmonary resections show different results. Severe oxidative damage to proteins was found among patients undergoing pneumonectomy – a procedure without release of reoxygenation metabolites [6]. In some individuals, during OLV, the non-operated lung may be hyperinflated with high concentrations of oxygen, which could be an additional, constant intraoperative source of ROS. Two patients in group I underwent pneumonectomy. These patients did not suffer from re-expansion flush of hypoxemic, ROS rich blood. We decided not to exclude these patients as they underwent long lasting OLV with constant release of blood through a pulmonary shunt. Our study does not reveal significant differences between intraoperative changes of protein peroxidation derivatives. Both ischemia and reperfusion together with ventilatory reoxygenation may be responsible for the exaggerated oxidative stress.

Oxidative damage to proteins may lead to serious tissue dysfunctions. In the present study a significant intraoperative decrease in protein concentration was found in the OLV group. Oxidative stress is involved in the pathomechanism of ALI or ARDS. In the hypoxemic, hypoventilated and hypoperfused lung, high amounts of ROS are released. High ROS reaction rate constants result in their very local action, which could significantly impair pulmonary vessels of the endothelium. Nevertheless, in the postoperative period, ARDS and ALI do not occur more frequently in patients undergoing OLV. This study did not evaluate intraoperative pulmonary endothelium impairment. It is likely that the degree of pulmonary edema after OLV may be clinically important due to postoperative atelectasis in circulatory compromised patients [4, 10, 11]. The function of pulmonary vessels' endothelium during and after OLV still needs to be clarified.

Atrial fibrillation was more common in the OLV when compared to the control group. However, the difference

was not significant. The changes in intraoperative and postoperative concentrations of oxidative derivatives did not significantly influence the rate of atrial fibrillation. OLV longer than 60 minutes is considered to be a potential risk factor of atrial fibrillation in the postoperative period after lung resection [5]. It is likely that due to the shorter period of OLV in this study, the previously described differences were not observed. Rates of other postoperative complications did not significantly differ between study groups.

In conclusion, OLV is an independent risk factor of increased oxidative stress. No influence of oxidative stress generated during a short period of OLV on the complication rate after lung resection was observed.

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