Alterations in bronchial nitric oxide release and pulmonary function after cardiopulmonary bypass in patients with normal and decreased respiratory capacity

Normal ve azalmış respiratuvar kapasiteli kardiyopulmoner bypass hastalarında pulmoner fonksiyon ve bronşiyal nitrik oksit salınımındaki değişimler

Şahin Şenay, Gökçen Orhan, Ayliz Velioğlu,[1] Batuhan Özay, Murat Sargın, Müge Taşdemir, Okan Yücel, Erol Kurç, Serap Aykut Aka

Department of Cardiovascular Surgery, Dr. Siyami Ersek Thoracic and Cardiovascular Surgery Center; [1]Department of Public Health, Medicine Faculty of Marmara University, İstanbul

Background: The aim of this study is to evaluate the pulmonary function and define the alterations in bronchial nitric oxide (NO) release in correlation with the vascular endothelial NO release in patients with normal and decreased respiratory capacity undergoing coronary artery bypass graft surgery under cardiopulmonary bypass.

Methods: Twenty patients with normal pulmonary functions and 20 patients with decreased respiratory function were involved in the study. Endotracheal aspiration samples and plasma samples were obtained just after the entubation, at the end of the cardiopulmonary bypass and at the postoperative sixth hour. The respiratory functional status, bronchial NO release and vascular NO release were evaluated.

Results: The NO levels in plasma and bronchial samples, and respiratory capacity were gradually decreased; and respiratory index values significantly deteriorated in both groups.

Conclusion: These findings show that patients with chronic obstructive lung disease undergoing cardiopulmonary bypass have similar lung damage with patients who have normal respiratory capacity.

Key words: Cardiopulmonary bypass; coronary artery bypass; lung volume measurements; nitric oxide.

Amaç: Bu çalışmanın amacı normal ve azalmış respiratuvar kapasiteli, aorta koroner bypass cerrahisi ve kardiyopulmoner bypass geçiren hastalarda pulmoner fonksiyonları ve vasküler endotelyal nitrik oksit (NO) salınımı ile birlikte bronşiyal NO salınımındaki değişimleri değerlendirmektir.

Çalışma planı: Yirmi normal pulmoner fonksiyonlara sahip hasta ile 20 azalmış pulmoner fonksiyonlu hasta çalışmaya dahil edildi. Entübasyondan hemen sonra, kardiyopulmoner bypass sonrası ve ameliyat sonrası altıncı saatte endotrakeal aspirasyon ve plazma örnekleri alındı. Respiratuvar fonksiyon durumları, bronşiyal ve vasküler NO salınımları değerlendirildi.

Bulgular: Her iki grupta da, plazma ve bronşiyal NO salınımı ve respiratuvar kapasite kardiyopulmoner bypass sonrası yavaşça azaldı; respiratuvar indeks değerleri önemli derecede kötüleşti.

Sonuç: Bu bulgular, kardiyopulmoner bypass'a girecek olan kronik obstrüktif akciğer hastalığı bulunanların, normal respiratuvar kapasiteli hastalarla benzer akciğer hasarına maruz kaldıklarını göstermiştir.

Anahtar sözcükler: Kardiyopulmoner bypass; koroner arter bypass; akciğer kapasitesi ölçümü; nitrik oksit.

Cardiopulmonary bypass (CPB) may cause pulmonary dysfunction and endothelial injury. Activation of the complement system, endotoxemia, ischemia and reperfusion injury, and surgical trauma are all potential triggers of inflammation following CPB. The major mechanisms of this inflammation is linked to the transient and incomplete lung ischemia associated with pulmonary arterial blood flow diversion during CPB, followed by reperfusion of the pulmonary vascular bed and systemic inflammatory response.[1] A significant consequence of reperfusion injury is dysfunction of the pulmonary vascular endothelium with secondary vasoconstriction and increased vascular permeability leading to pulmonary hypertension, pulmonary edema and hypoxia.[2,3]

Received: 5 Ocak 2007 *Accepted*: 18 Nisan 2007

Correspondence: Dr. Batuhan Özay. Dr. Siyami Ersek Göğüs Kalp ve Damar Cerrahisi Eğitim ve Araştırma Hastanesi, Kalp ve Damar Cerrahisi Kliniği, 34668 Haydarpaşa, İstanbul. Tel: 0216 - 456 54 44 e-posta: drbatuhanozay@yahoo.com

This may result in an adverse clinical outcome especially in the patients with limited respiratory capacity.

Nitric oxide (NO) plays a significant role in both the normal and pathologic physiology of lung. In the lung, NO is an important regulator of intercellular interaction, affecting airway and microvascular reactivity and permeability. Pulmonary vascular endothelial cells synthesize NO after certain stimuli, such as shear stress and the receptor binding of specific vasodilators, which finally activate endothelial NOS (eNOS). Endogenously produced endothelium-derived NO is an important mediator of normal pulmonary physiological functions. Any aberrations in basal NO production may be implicated in the pathophysiology of postoperative pulmonary dysfunction.[4,5]

The overall underlying mechanisms of this process have not been entirely elucidated yet.[4] There is a dilemma in the postoperative physiopathology of pulmonary NO release. Decreases in NO activity have been demonstrated after CPB in some studies. While some other several reports highlight increased activity of NO.

This study was designed in the aim to evaluate the correlation of alterations in bronchial and vascular endothelial NO release with pulmonary functions in patients with normal and decreased pulmonary capacity, undergoing coronary artery bypass graft (CABG) operation with CPB.

PATIENTS AND METHODS

Forty patients undergoing elective coronary bypass operations with CPB were enrolled in this study. Coronary artery disease patients under 65 years old, all indicated for primary CABG operation were selected. The patients had stable angina pectoris without any hemodynamic, enzymatic and electrocardiographic changes in the last 30 days. All patients had good ventricular function with ejection fraction >45%. None of the patients had a systemic inflammatory disease nor were receiving immune suppressive drugs.

The patients were grouped into two. Group 1 included the patients with normal pulmonary functions preoperatively. Group 2 included the patients with decreased respiratory capacity (2003 GOLD -Global Initiative for Chronic Obstructive Lung Disease- Classification Stage 2; FEV1/FEVC <%70, %50 <FEV1 <%80, symptomatic or asymptomatic patients) preoperatively.[6]

The study was approved by the local ethics committee of our institution and informed consent was obtained from all patients.

Routine CABG protocol of our cardiovascular surgery clinic was applied to all patients.

Anesthesia was induced with 20 mcg/kg fentanyl cytrate, 0.12 mg/kg pancuronium, 2 mg/kg propofol IV. After tracheal intubation, mechanical ventilation was instituted with 1.0 FiO_2, 6-8 ml/kg tidal volume, and 10-12 min respiratory rates. Anesthesia was maintained with 10 mcg/kg/hour of fentanyl, 1 mg/kg/hour propofol as infusion. 2 mg of pancuronium were given every 45 minutes through the operation.

After preparation of left internal mammary artery flap and greater saphenous vein grafts, 400 IU/kg heparin was administered before the institution of CPB. Activated clotting time was managed to be over 400 seconds during cardiopulmonary bypass. At the end of the CPB the heparin effect was reversed with protamine sulphate at 1:1 ratio.

Aortic and two-stage venous cannulae were used to institute the CPB using a roller pump, membrane oxygenation and identical priming solution. The content of prime solution was 1000 ml Ringer's lactate, 150 cc mannitol, 60 cc bicarbonate and 1 mg/kg heparin. Systemic blood flow was maintained at 2.2-2.4 L/m^2, mean arterial blood pressure at 60-70 mmHg during CPB. Systemic hypothermia (28 °C) and hemodilution were applied.

For myocardial protection, antegrade +4 ºC cold blood cardioplegia (1000 cc blood, 70 ml citrate, 750 mg magnesium sulphate, 3 mEq potassium/100 cc blood, 10 mEq Na-bicarbonate) was given 10 ml/kg at the beginning of the arrest and then repeated every 20 minutes. Topical cooling was maintained with cold saline solution.

Distal anastomoses were performed during the cross-clamp period. Proximal anastomoses were performed with partial occluding clamp on beating heart. All the left anterior descending arteries received pediculated left internal mammary artery grafts. The other vessels received greater saphenous vein grafts.

Postoperatively, pharmacological support was instituted according to hemodynamic requirements.

Evaluation of the respiratory capacity and function. The patient's respiratory functional tests for the measurement of the respiratory capacity were performed the day before the operation and at the postoperative seventh day. FEVC, FEV1, FEV1/FVC were measured. The respiratory index (RI= $AaDO_2/PaO_2$), which is used as one of the main determinants of the oxygen transport through the arterioalveolar membrane, was calculated by arterial blood gas assay pre-CPB, post-CPB and at the postoperative sixth hour to define post-bypass lung damage. This factor reflects the arterioalveolar oxygen gradient and its level increases due to any impairment of oxygenation through this membrane. The parameters

of this calculation are obtained from arterial blood gas analysis.

$RI = AaDO_2/PaO_2$

$AaDO_2 = PAO_2 - PaO_2$

$PAO_2 = PIO_2 - PACO_2 \times [(FIO_2 + (1 - FIO_2/R)]$

$PIO_2 = (760 - PH_2O) \times FIO_2$

RI: Respiratory index; $AaDO_2$: Arterioalveolar oxygen gradient; PaO_2: Arteriolar oxygen pressure; PAO_2: Alveolar oxygen pressure; PIO_2: Inspiration oxygen pressure; $PACO_2$: Alveolar carbondioxide pressure; FIO_2: Inspiratory fraction of oxygen; PH_2O: Water pressure.

Sample collection. Blood serum, arterial gas and mixed venous samples were taken preoperatively and repeated for follow up parameters in the postoperative period at admission to intensive care unit (ICU) and at the sixth postoperative hour. Systemic blood samples (10 cc) for serum NO detection were taken with the start of cardiopulmonary bypass, after the CPB, and at postoperative sixth hour in the ICU.

Maintaining endotracheal aspiration (ETA) samples. 5 cc of ETA was taken from the endotracheal tube, in order to detect NO levels. First samples are taken after anesthesia induction; second samples are taken at the end of CPB and the third ones at the postoperative sixth hour.

NO determination. All samples were concentrated by centrisart-1 tubes (cut-off 10.000, Sartorius, Goettingen-Germany) and storaged at –20° C. Before concentration by centrisart-1, all aspiration samples were dissolved in 0.1% DDT (dithiothreitol). After incubation at 37 ºC, they were mixed with PBS (pH 7.2). We used in our investigation Nitric Oxide Colorimetric Assay (Roche Molecular Biochemical's, Mannheim-Germany) to determine the NO levels in serum and bronchial aspiration samples. In biological fluids NO is very rapidly deactivated by oxidation to nitrite and nitrate by physically dissolved oxygen and water. In NO colorimetric assay, NO levels are determined photometrically via its oxidation products nitrite and nitrate. In biological fluids, NO is measured via nitrite. In our test procedures, nitrate present in sample, is reduced to nitrite by reduced NADPH in presence of the enzyme nitrate reductase. Nitrite levels can be measured as diazo dye in the visible range at 550 nm.[7]

Table 1. Demographic variables

	Group 1	Group 2	*p*
Mean age (years)	61.8±4.32	59.6±5.48	NS
Male patients (n)	6	7	NS
Diabetes mellitus (n)	3	5	NS
Hypertension (n)	4	5	NS
Previous MI	3	3	NS
Ejection fraction %	64.6+20.11	66.7+15.7	NS
Hyperlipidemia (n)	7	8	NS
Smoking (n)	6	7	NS
Body mass index	27.3+2.7	26.5+3.2	NS

Group 1: Normal pulmonary function preoperatively; Group 2: Pulmonary dysfunction preoperatively; MI: Myocardial infarction; EF: Ejection fraction; BMI: Body mass index; NS: Non significant.

Statistical analysis. All the results are given as mean±standard deviation from the mean and percentages. Study groups were compared by using either student t-test or non-parametric Mann Whitney-U when the data did not follow Gaussian distribution. In comparison of categorical data chi square and Fisher exact tests were used when they were appropriate. A repeated measure ANOVA was used to assess changes in respiratory index, serum nitric oxide levels and endobronchial nitric oxide levels during preoperative and postoperative period. A $p<0.05$ was considered significant. SPSS for windows statistical software program was used in all statistical comparisons.

RESULTS

The demographic and clinical data of the patients are given in Table 1. There were no statistical differences among the preoperative variables of the patients. The

Table 2. Perioperative data of the patients

	Group 1	Group 2	*p*
Number of distal anastomoses	2.60±0.5	2.7±0.50	NS
Number of proximal anastomoses	1.60±0.5	1.70±0.5	NS
CPB time (minutes)	65.30±9.1	67.5±11.5	NS
Aortic cross clamp time (minutes)	45.00±6.46	47.00±8.76	NS
Blood transfusion (units)	2.30±0.48	1.90±0.57	NS
Mechanical ventilation (hours)	10.9±1.20	14.5±1.27	<0.05
Postoperative atrial fibrillation	1	3	NS
Stay in the intensive care unit (hours)	22.6±1.84	24.2±1.75	NS
Stay in the hospital (days)	7.3±0.48	7.7±0.42	NS
Mortality	0	0	NS

CPB: Cardiopulmonary bypass; NS: Non significant.

Table 3. FEV1/ FVC measurements

	COLD*	Control*
Basal levels	92.31±3.28	60.73±5.38
Postoperative 7th day levels	90.12±2.48	63.23±4.71

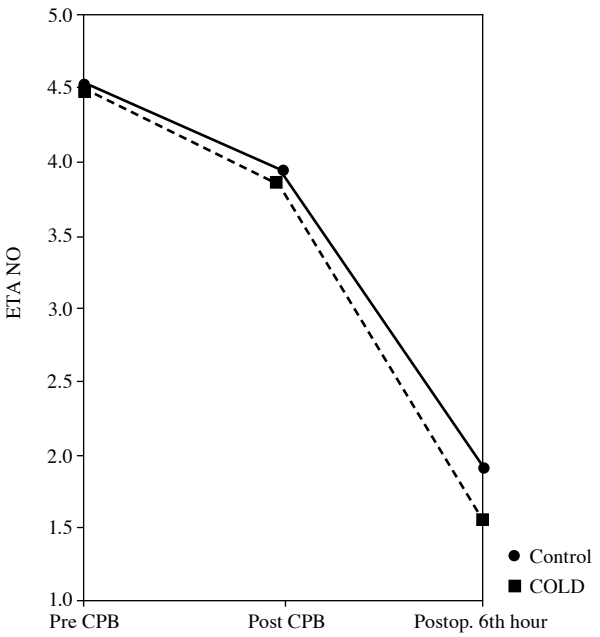
*: p<0.05 between groups, no significant change was determined within the groups; COLD: Chronic obstructive lung disease.

perioperative variables of the patients were similar (Table 2). Basal FEV1/FVC measurements of group 2 were significantly lower (group 1 vs group 2: 92.31±3.28 vs 60.73±5.38) (Table 3).

The NO levels were decreased gradually and significantly in serum and ETA samples in both groups. The change in the serum and the bronchial NO levels were correlated (Table 3) (Fig. 1, 2).

Respiratory index before CPB was similar between the groups (group 2 vs 1: 0.41±0.01 vs 0.38±0.01), and there were significant changes in the second and the third measurements in RI value in both groups (p<0:0001; ANOVA, treatment effect) (Fig. 3).

Duration of mechanical ventilation was significantly longer in group 2 (Table 2).



	COLD*	Control*
Pre CPB**	4.46±0.47	4.51±0.47
Post CPB**	3.8±0.39	3.9±0.39
Postop. 6th hour**	1.5±0.15	1.9±0.15

*: p=0.68 between groups; **: p<0.001 within COLD and control groups.

Fig. 1. Bronchial NO levels. Endotracheal NO levels (Mean values±SEM); COLD: Chronic obstructive lung disease; CPB: Cardiopulmonary bypass; Values as micro M/L.

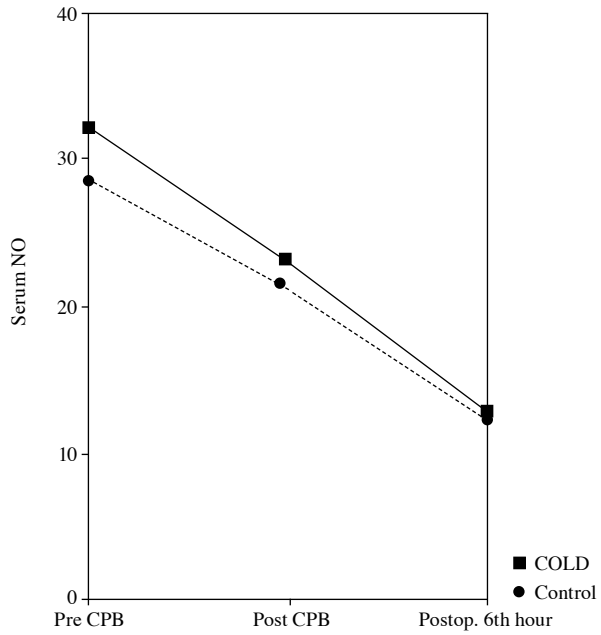
Postoperative seventh day measurements of FEV1/ FVC were found to be decreased slightly (group 1 vs group 2: 90.12±2.48 vs 63.23±4.71) and the values were significantly lower in group 2.

DISCUSSION

Diffuse systemic inflammatory responses during and after cardiac surgery are primarily related to cardiopulmonary bypass. These complex inflammatory responses are mediated by complement, cytokine, and kininogen/ bradykinin pathways and are intimately linked to the coagulation cascade and fibrinolysis.[8,9] Notably, the patients with decreased pulmonary function are prone to lung injury due to the inflammatory response in coronary surgery. At the postoperative period different types of complications, especially prolonged intubation time and acute respiratory insufficiencies can be seen in this group of patients.[10]

Although the cellular and molecular events underlying the pathological response to heart surgery are not yet entirely clear, NO is known to be involved and could serve both to mediate and indicate lung injury in cardiac surgery with CPB.[11,12]

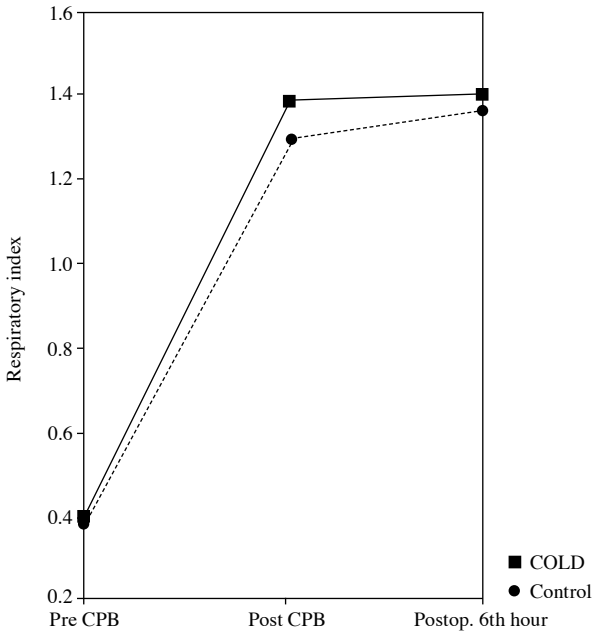
The NO production is regulated by endothelial-NOS (eNOS) and inducible NOS (iNOS) in the development



	COLD*	Control*
Pre CPB**	32.45±1.89	28.63±1.89
Post CPB**	22.33±1.9	20.71±1.9
Postop. 6th hour**	11.12±0.62	10.78±0.62

*: p=0.31 between groups; **: p<0.001 within COLD and control groups.

Fig. 2. Serum NO levels (Mean values±SEM); COLD: Chronic obstructive lung disease; CPB: Cardiopulmonary bypass; Values as micro M/L.



	COLD*	Control*
Pre CPB**	0.41±0.01	0.38±0.01
Post CPB**	1.38±0,04	1.27±0.04
Postop. 6th hour**	1.40±0.04	1.35±0.04

*: p=0.54 between groups; **: p<0.001 within COLD and control groups.

Fig. 3. Respiratory index levels (Mean values±SEM); COLD: Chronic obstructive lung disease; CPB: Cardiopulmonary bypass; FEV1: Forced expiratory volume in first second; FVC: Forced vital capacity; RI: Respiratory index; CPB: Cardiopulmonary bypass.

of CPB-induced inflammatory response. The ETA specimens are compounds of endothelial secretions through the lung, the NO levels of these specimens may reflect the EC dysfunction. The NO levels in the ETA sample can be determined as a marker of bronchial endothelial function and can differ according to the degree of inflammatory process altering the endothelial function in response to inflammatory reaction of lung tissue.[7,13,14]

The basal serum and ETA levels of NO groups were not significantly different but post cardiopulmonary bypass levels showed significance. There was a correlation between vascular and bronchial release of NO levels. The NO levels in both patient groups decreased gradually.

The RI values were found to be increased threefold after cardiopulmonary bypass. The comparison of increase between two groups showed no significance. The RI values at postoperative sixth hour were also high but similar to post cardiopulmonary bypass levels.

The change of vascular and brochial NO levels and RI values may reflect the inflammatory effect of CPB on respiratory physiology. The insignificant values between groups are worth for discussion. Although the patients included in the study as group 2 had decreased respiratory capacity preoperatively, (2003 GOLD-Global Initiative for Chronic Obstructive Lung Disease-Classification Stage 2), the CPB seems to have a similar effect on both groups. The CPB effect on respiratory functions may be diverse in patients with more severely decreased respiratory capacity.

However, previous studies of the changes in basal NO production induced by CPB, as determined on the basis of plasma levels of NO metabolites, have been inconsistent. Despite extensive research into both pro-inflammatory and anti-inflammatory actions of NO, the overall contribution of NO to inflammatory conditions of the lung is not easily predicted and seems to depend on many factors, such as the site, time and degree of NO production in relation to the local redox status, and the acute or chronic nature of the immune response.[15,16]

In the respiratory tract, NO is generated enzymatically by three distinct isoforms of NO synthase (NOS-1, NOS-2 and NOS-3) that are present to different extents in numerous cell types, including airway and alveolar epithelial cells, neuronal cells, macrophages, neutrophils, mast cells, and endothelial and smooth muscle cells. Inflammatory diseases of the respiratory tract, such as asthma, acute respiratory distress syndrome (ARDS) and bronchiectasis, are commonly characterized by an increased expression of NOS-2 within respiratory epithelial and inflammatory-immune cells, and a markedly elevated local production of NO, presumably as an additional host defense mechanism against bacterial or viral infections. Pro-inflammatory cytokines and endotoxin can induce the release of NO by EC and smooth muscle cells through the inducible form of the enzyme NOS (iNOS). Constitutive NO (cNO) is normally produced by EC from the amino acid L-arginine by means of calcium-dependent NOS. Nitric oxide modulates vasomotor tone in response to physiologic stimuli such as pulsatile flow and shear stress.[17-20]

In conclusion, we evaluated that, there is a bronchial and vascular NO release impairment after CPB, and this is correlated with the respiratory capacity. This may reflect the bronchial endothelial cell dysfunction after CPB. Nevertheless, RI values and NO release are similarly affected during CPB. The patients with COPD at stage 2 can be operated with COPD under similar risks in terms of decrease in respiratory capacity. Further studies may help the status for patients with more severe COPD.

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