**Dynamic changes in blood concentrations of nitrite / nitrate
and methemoglobin in patients after lung transplantation during
treatment with inhaled nitric oxide**

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Understanding of the nitric oxide (NO) role in the pathogenesis of acute and chronic disorders of the pulmonary circulation contributed to the introduction of inhaled NO (iNO) in the complex therapy of patients after lung transplantation. Inhaled NO can form methemoglobin (MetHb) and nitrate. The aim of the study was to investigate the relationship between iNO and nitrite/nitrate (NOx) and MetHb levels in the blood serum of 7 patients after lung transplantation. Duration of iNO therapy was 3 ± 1 days, the data were reviewed at 1 to 10 days after transplantation. Patients showed a significant increase of the NOx levels from day 1 to 5 after surgery, meanwhile the MetHb levels remained within the normal range. The significant positive correlations were found between the iNO use and NOx concentrations, between iNO use and MetHb levels, and between NOx and MetHb levels. The obtained results suggest that the iNO use in lung transplant recipients requires a close monitoring of the administered iNO concentration and the blood levels of MetHb and NOx.

Key words: lung transplantation, inhaled nitric oxide, nitrite/nitrate, methemoglobin.

Introduction

Endogenous nitric oxide (NO) is a vasodilator synthesized from L-arginine by NO-synthase and acting upon the endothelial lining of blood vessels. NO is involved in the regulation of the systemic and pulmonary vascular resistance. Under normal conditions, NO is predominantly produced by endothelial NO-synthase (eNOS) [1, 2]. An alternative NO-synthase isoform, namely inducible NO-synthase (iNOS), is stimulated by cytokines, bacterial endotoxins and can produce excessive amounts of NO during inflammation or stress [2, 3].

Understanding of the role played by the pulmonary vascular endothelial dysfunction and NO synthesis disorders in the pathogenesis of acute and chronic pulmonary circulation disorders contributed to the introduction of inhaled NO (iNO) as an exogenous analogue of a natural regulator of vascular tone in a complex therapy of patients after lung transplantation (LT) [4].

During LT, the main role of iNO is believed to belong to its impact on the pulmonary vascular resistance. Meanwhile, in pulmonary hypertension, iNO may reduce the pulmonary vascular resistance without changes in the systemic vascular resistance, and improve oxygenation by dilating the blood vessels in the ventilated areas of the lungs reducing the shunt fraction [5-7]. Moreover, iNO probably has a beneficial effect after lung transplantation attenuating the reperfusion injury of the endothelium and reducing a primary graft failure.

The administration of iNO in low concentrations seems safe. In high concentrations, however, its toxicity is predominantly related to NO_2 production and methemoglobinemia. Interaction of iNO with oxyhemoglobin results in the production of methemoglobin (MetHb) and nitrite/nitrate (NOx) [8]. Almost 70% of iNO is excreted in urine within 48 hours in the form of nitrates [9]. Excessive concentrations of circulating MetHb can cause a tissue hypoxia [10]. MetHb is not capable of binding oxygen, so MetHb production may induce a hemic hypoxia. Moreover, in the presence of MetHb, the oxyhemoglobin dissociation curve is shifted to the left resulting in a decreased oxygen delivery to tissues. That is why the inhaled NO administration demands a close monitoring of blood MetHb levels. However, there are very few studies to investigate blood NOx levels in the lung transplant recipients in whom the inhaled NO was administered. Therefore, the aim of this study was to investigate the relationship between the iNO administration and the blood MetHb and NOx levels in patients after LT.

Materials and methods

The study included 7 patients (6 females and 1 male) aged between 24 and 55 (36.3 ± 4.0) years after lung transplantation. Lung transplantations were performed for primary pulmonary hypertension (2 cases), cystic fibrosis (2 cases), lymphangioleiomyomatosis, sarcoidosis, and idiopathic pulmonary fibrosis (1 case each).

Mechanical lung ventilation (MLV) was provided in an individually parameter-selected mode using Primus devices (in the Operating Room) and EvitaXL devices (in ICU). Ventilator settings and lung biomechanics were recorded by a monitoring system in a real time.

The inhaled NO therapy was conducted using commercially available gas mixture NO-NO$_2$ with the NO concentration of 1000 ppm (parts per million). The iNO (5-20 ppm) was delivered into the inspiratory limb of the breathing circuit of the ventilator system at 60-80 cm away from the Y-piece. A low gas flow rate was ensured using the Bedfont Nitric Oxide Inhaled Therapy Flow system. An iNO volumetric flow rate (ml/min) was set up and adjusted according to a required concentration and the readings of the electrochemical NO-NO$_2$ analyzer. Duration of iNO-therapy ranged from 2 to 4 days, the mean iNO concentration was 30±1 ppm.

Measurements of serum NOx were performed using the reduction reaction of nitrate into nitrite with cadmium in the presence of zink. [11]. Acid-base status, blood gas, and arterial MetHb were assessed using the ABL 800 Flex blood gas analyzer (Radiometer, Denmark). The measurements were made from day 1 to day 10 post-surgery. The control group included 25 healthy volunteers, mean age 32.7 ± 8.6 years; with male/female ratio 17/8.

The statistical analysis was performed using Statistica 10.0 and MS Excel Package Software. The comparisons between the study group and the control group were made using Mann-Whitney U-test. Spearman rank correlation was used to test the association between variables. The data were expressed as medians and interquartile range (25th and 75th percentiles).

Results and discussion

Studies of the blood NOx and MetHb levels in patients after LT over time demonstrated a statistically significant increase of NOx levels and 2.5 times on days 1, 2, 3, 4, and 5 after surgery, respectively (Table. 1). Graphs of NOx levels changing over time individually for each patient after LT are

presented in the Figure demonstrating that blood serum NOx levels exceeded normal range in all the patients for 5 days following surgery, later on gradually decreasing in 6 patients, and nearly reached the normal values at day 10. MetHb levels remained within normal rage (Table. 2).

Table 1. Changes of blood NOx and MetHb levels over time in patients after lung transplantation (data are presented as medians and interquartile ranges).

Parameter	Normal value	Day						
		1st	2nd	3rd	4th	5th	7th	10th
NOx, mcmol/L	18.61 (17.70-23.62)	67.73 * (50.48-87.30)	72.05 * (71.02-103.63)	79.49 * (63.87-112.80)	54.98 * (52.78-91.09)	45.77 * (23.49-91.75)	13.37 (11.69-110.29)	17.38 (15.11-44.94)
MetHb,%	1.3 (1.0-1.4)	1.5 (1.3-1.5)	1.5 (1.4-1.7)	1.0 (1.0-1.2)	1.1 (0.9-1.3)	1.0 (0.8-1.1)	0.7 (0.7-0.9)	0.8 (0.7-1.0)

* P <0.05 compared to normal.
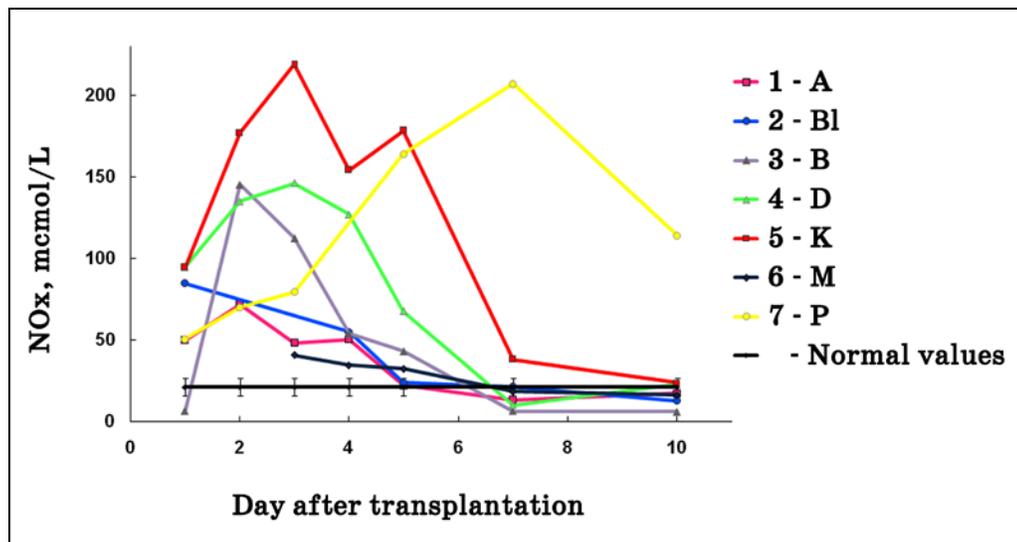
Figure. Dynamics of NOx levels in patients after transplantation

Our data are consistent with the data presented by W.Steudel et al. who showed that a significant methemoglobinemia or NO_2 production were

rare events in the patients on iNO therapy in doses less than or equal to 80 ppm [12]. Passing through an alveolar-capillary membrane, iNO may interact with oxygen producing NOx, or bond with oxyhemoglobin to produce not only NOx, but also MetHb. Considering iNO metabolism in a human body, we studied the relationships between iNO administration and the whole blood MetHb level, and serum NOx level in patients after LT.

In the total study group of patients after LT we observed the following statistically significant positive correlations: between the iNO administration and NOx levels, between the iNO administration and MetHb levels, between the levels of NOx and MetHb (Table 2). These data suggest an ongoing iNO metabolism in the body with the production of NOx and MetHb.

Table 2. Correlations between the iNO administration and NOx and MetHb levels in a group of patients after lung transplantation.

Parameter	iNO	NOx	MetHb
NOx	R=0.866, P=0.012 (N=7)	-	R=0.870, P =0.002 (N = 4)
MetHb	R=0.840, P=0.036 (N=4)	R=0.870, P=0.002 (N=4)	-

Furthermore, our studies showed statistically significant positive correlations individually for each patient after LT:

- between the iNO administration and NOx levels in patients 2, 3, 4 (Table. 3);

- between the iNO administration and MetHb level in patient 4 (Table. 3);

- between NOx levels and MetHb levels in patients 1, and 4 (Table.3).

Table 3. Correlations between the iNO administration, NOx levels and MetHb levels in patients after transplantation.

Patients	Correlation between		
	iNO and NOx	iNO and MetHb	NOx and MetHb
1 – A	R = 0.545, P = 0.129	R = 0.521, P = 0.150	R = 0.720, P = 0.029
2 - Bl	R = 0.828, P = 0.042	R = 0.105, P = 0.843	R = 0.174, P = 0.742
3 - B	R = 0.779, P = 0.013	-	-
4 - D	R = 0.853, P = 0.002	R = 0.880, P = 0.0008	R = 0.900, P = 0.0004
5 - K	R = 0.293, P = 0.573	-	-
6 - M	R = 0.393, P = 0.441	-	-
7 - P	R = -0.693, P = 0.039	R = 0.044, P = 0.911	R = -0.328, P = 0.389

The correlations observed for individual patients also confirm the fact that iNO is metabolized in the body to produce predominantly NOx.

Given the increasing number of LTs performed annually worldwide, enhanced studies are necessary to investigate the NO metabolism in the blood serum of the patients after successful LT and those who experienced an acute or chronic graft rejection. There are only a few reports on the studies to have investigated the levels of stable NO metabolites in patients after LT. So, De Andrade et al. [13] reported a significant increase of the total NOx concentrations in bronchoalveolar lavage (BAL) fluid and in the blood serum of the patients after LT compared to those of control subjects. These authors also demonstrated the correlation between serum NOx levels in the patients and the degree of airway inflammation, and fibrosis due to the transplant rejection [14]. Reid et al. [15] confirmed the findings of increased nitrite levels in patients after LT, however, stratifying the patients into the stable group and the group of those with bronchiolitis obliterans, they observed a significantly increased nitrite level in the BAL fluid in the latter group only. Increased circulating nitrite levels were reported in experimental studies in animals with acute cellular rejection of the graft [16, 17].

In our study, a statistically significant increase of the NOx level, approximately by 3-4 times, was observed in patients at 1-5 days after LT; and a significant positive correlation between the iNO administration and NOx levels was also found. The significant increase in serum NOx in patients at 1-5 days after LT, might be related to iNO metabolism and its transformation to NOx, and also to the activation of iNOS. Further studies of this issue are necessary.

Conclusion

The obtained results suggest that the iNO use in lung transplant recipients requires a close monitoring of the administered iNO concentration and the blood levels of MetHb and NOx.

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