Plasma Levels of Nitric Oxide in Children with Congenital Heart Disease and Increased Pulmonary Blood Flow

Patchara Kiettisanpipop MD*, Pornthep Lertsapcharorn MD*,
Pairoj Chotivittayatarakorn MD*, Yong Poovorawan MD*

** Department of Pediatrics, Faculty of Medicine, Chulalongkorn University*

Background: *Development of pulmonary hypertension commonly accompanies congenital heart disease; nitric oxide (NO) is evidently an important mediator of pulmonary vascular reactivity.*
Objective: *Investigate the effect of pulmonary hypertension (PH) associated with congenital heart disease on NO production.*
Material and Method: *The authors measured plasma levels of nitric oxide-related compounds in 28 patients, aged 3 months to 12 years with congenital heart disease (CHD) and increased pulmonary blood flow. Blood samples were obtained during their cardiac catheterization. The subjects were subsequently divided into two groups, namely: group 1 CHD were those with left-to-right shunt; and group 2, CHD with right-to-left shunt.*
Results: *Four patients had severe pulmonary hypertension (mean pulmonary arterial pressure > 60 mmHg). The total levels of NO-related compounds between the two groups were not statistically different as well as the levels in pre- and post-pulmonary artery. In patients with left-to-right shunt with mild to moderate pulmonary hypertension, the levels of total NO-related compounds were directly correlated with the level of pulmonary arterial pressure and pulmonary vascular resistance ($r = 0.67$; p-value < 0.05, and $r = 0.75$; p-value < 0.05). Additionally, in patients with severe pulmonary hypertension, the levels of total NO-related compounds decreased when compared to the levels in patients with mild to moderate pulmonary hypertension.*
Conclusion: *The present results suggested that the hemodynamic status of the pulmonary circulation in congenital heart defect is at least partly correlated with the blood levels of nitric oxide.*

Keywords: *Plasma nitric oxide, Pulmonary arterial pressure, Congenital heart disease*

J Med Assoc Thai 2007; 90 (10): 2053-7
Full text. e-Journal: *http://www.medassocthai.org/journal*

Development of pulmonary hypertension (PH) commonly accompanies congenital heart disease with increased pulmonary blood flow. Recent evidence suggests that pulmonary vascular endothelium is an important determinant of the vascular tone and it has been hypothesized that endothelial injury secondary to increased pulmonary blood flow and/or pressure plays a role in the development of PH[1,2]. Nitric oxide (NO) is an endothelium-derived relaxing factor, synthesized and released from the endothelial cells by the activation of endothelial nitric oxide synthase (eNOS). It induces vascular smooth muscle relaxation through the activation of a cGMP-dependent protein, kinase and it plays long-term effects on fibro-production and reparative vascular remodeling process[3]. Plasma NO increases in patients with congenital heart disease with left-to-right shunt and the levels decrease after surgical correction[4-11]. In the present study, the authors investigated the relationship between plasma NO levels and the degree of pulmonary arterial pressure in patients with congenital cardiac defects with increased pulmonary blood flow both with cyanotic and acyanotic cardiac lesions.

Correspondence to : Poovorawan Y, Center of Excellence in Viral Hepatitis Research, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. Phone: 0-2256-4909, Fax: 0-2256-4929, E-mail: Yong.P @chula.ac.th

Material and Method

Population study

The authors studied children who had cardiac lesions with increased pulmonary blood flow from the age of 3 months to 12 years. They had no significant

underlying diseases such as metabolic disorders, renal disease, and infection; and they were admitted to the Pediatric Cardiology Unit, Chulalongkorn Memorial Hospital for cardiac catheterization.

Blood samples were obtained from the right ventricle or pulmonary artery, which represents pre-pulmonary arterial blood, and the left atrium or left ventricle, which represents post-pulmonary arterial blood. The present study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. The parents of all subjects were fully informed about the objective of the study and written consent was obtained from the parents of each subject before blood samples were collected.

Measurement of plasma nitric oxide related compounds

Blood samples were collected via EDTA tube and centrifuged within six hours. The plasma samples were frozen below -20°C until assay were performed. All samples required at least a 2-fold dilution and ultra filtered through a 10,000 molecular weight cutoff filter to eliminate proteins. All NO-related compounds (nitrate and nitrite) were determined as a colored azo-dye production of the Griess Reaction via R&D Systems (Saliva Sac□, Pacific Biometric Inc, Seattle, USA). Nitric Oxide Assay measured by a spectrophotometer following the manufacture's instructions. Specific standard curve of nitrate and nitrite assays of the authors' setting was performed.

Statistical analysis

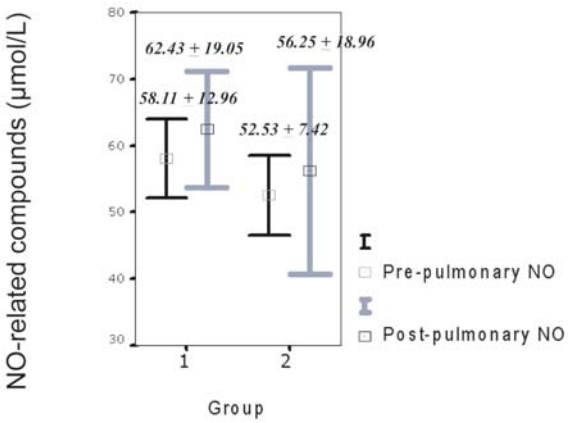
The data were analyzed using SPSS 11.5 computer software for Windows. The correlation between total plasma NO-related compounds level and other parameters were assessed using Pearson's correlation coefficient. Mean □ standard deviation (SD) plasma nitric oxide levels between the groups were compared using Student t-test. P-values of less than 0.05 were considered statistically significant.

Results

There were 28 patients recruited in the present study. Their ages ranged from 3 months to 12 years. The patients were subsequently divided into two groups on the basis of their hemodynamic characteristics, namely: group 1 (n = 21, 8 male and 13 female patients), the patients' cardiac lesions were left-to-right shunt that included ventricular septal defect, patent ductus arteriosus and complete AV canal defect; group 2 (n = 7, 4 male and 3 female patients), the patients' cardiac lesions were right-to-left shunt with increased pulmonary blood flow that included truncus arteriosus, single atrium, supracardiac total anomalous pulmonary venous return and double outlet right ventricle with ventricular septal defect. There were no differences between age or sex distribution of the two groups (group 1:5 mo-13 yrs, group 2:3 mo-10 yrs). In addition, all patients had normal serum creatinine levels. Eighteen patients received medical treatments including digoxin, angiotensin converting enzyme inhibitor, and diuretics for the control of heart failure. Four patients had severe pulmonary hypertension (mean pulmonary arterial pressure > 60 mmHg).

Pre- and post pulmonary artery blood of the patients with acyanotic cardiac lesions (group 1) had higher NO-related compounds levels (58.11 $\pm$ 12.96 umol/L and 62.43 $\pm$ 19.05 umol/L), compared to those with cyanotic cardiac lesions (52.53 $\pm$ 7.42 umol/L and 56.25 $\pm$ 18.96 umol/L). However, there was no detected statistical significance ($p = 0.330$ for pre-pulmonary, $p = 0.495$ for post pulmonary). Also, the levels of NO-related compounds of post pulmonary artery blood of both groups (62.43 $\pm$ 19.05 umol/L and 56.25 $\pm$ 18.96 umol/L, respectively) were higher than those of pre-pulmonary artery blood (58.11 $\pm$ 12.96 umol/L and 52.53 $\pm$ 7.42 umol/L, respectively), but there was no statistical significance (Fig. 1).

In acyanotic patients with mild to moderate pulmonary hypertension (mean pulmonary arterial pressure $\leq$ 60 mmHg and/or Rp/Rs < 0.5), post pulmonary artery blood levels of NO-related compounds had positive correlation with pulmonary arterial pressure



* *Italic* represent mean $\pm$ SD

Fig 1. Levels of NO-related compounds in group 1 (acyanotic patients) and group 2 (cyanotic patients)

(r = 0.67; p-value = 0.005) and pulmonary to systemic resistance ratio (r = 0.75; p-value = 0.001) but it had poor correlation with pulmonary to systemic blood flow ratio (Qp/Qs), and cardiac output or cardiac index. The post-pulmonary total NO-related markedly decreased in patients with severe pulmonary hypertension (MPA > 60 mmHg or Rp/Rs > 0.5) (Fig. 2, 3). Furthermore, the authors found the same correlation between nitric oxide and pulmonary arterial pressure (r = 0.693; p = 0.127) or pulmonary vascular resistance (r = 0.749; p = 0.087) in patients with cyanotic cardiac lesions.

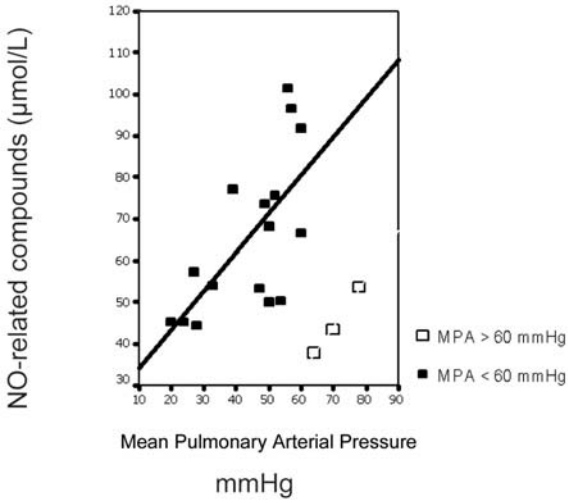


Fig 2. Relationship between NO-related compound level and MPA pressure in patients with left-to-right shunt; closed rectangle, MPA < 60 mmHg (R = 0.668, *p* = 0.005)

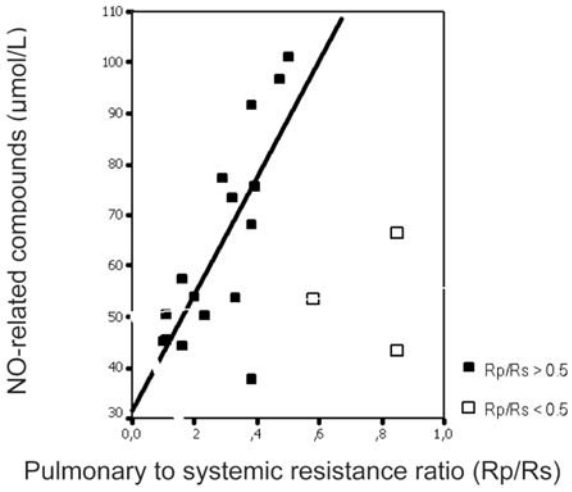


Fig 3. Relationship between NO-related compound level and pulmonary to systemic resistance ratio (Rp/Rs) in patients with left-to-right shunt; closed rectangle Rp/Rs < 0.5 (R = 0.753, *p* = 0.001)

Discussion

There are many factors contributing to pulmonary hypertension in patients with congenital heart disease and increased pulmonary blood flow. It may be partially related to the increase of pulmonary blood flow, which induces cyclic strain and shear stress on vascular endothelium[12]. Endothelial injury and the resulting alteration in endothelial function cause an imbalance between the productions of mediator which vasodilatation and vasoconstriction properties. The integrity of endothelium is essential for flow-mediated vasodilatation[13]. The flow-mediated vasodilatation is largely due to the release of NO, which is also known to modulate smooth muscle proliferation and growth. Nitric oxide is synthesized from various tissues including pulmonary vascular bed. The present study found that the levels of NO-related compounds at post-pulmonary artery site were slightly higher than that of the pre-pulmonary artery site; this may represent an important role of pulmonary vascular endothelium on nitric oxide synthesis.

Like other previous studies, the present study found that the level of plasma nitric oxide is elevated in patients with congenital heart disease with increased pulmonary blood flow, both in patients with acyanotic and cyanotic cardiac lesions. Plasma nitric oxide was correlated with pulmonary arterial pressure and pulmonary vascular resistance, and significantly decreased in patients with severe and long-standing pulmonary hypertension. These may represent the impairment of endothelium-dependent pulmonary vasodilatation in congenital heart disease with increased pulmonary blood flow. It is likely that the nitric oxide synthesis is elevated in earlier phases of pulmonary hypertension and reduced due to gross endothelial damage in advanced and terminal states[14-17]. Black SM and et al[18] found that shunted lambs displayed a selective impairment of endothelium-dependent pulmonary vasodilatation, suggestive of up-regulation of basal NO activity and gene expression in the early weeks of life, but they were further exposed to increased and/or pressure and resulted in progressive endothelial dysfunction and subsequent decrease in basal NO production.

In summary, NO activity participates in pulmonary artery hypertension, secondary to congenital

heart disease with increased pulmonary blood flow, and its up-regulation in mild and moderate pulmonary hypertension and decreased production in patients with advanced pulmonary vascular disease. Investigation of these changes and application, to predict the outcome of treatment strategies for patients with high pulmonary arterial pressure and low NO levels after surgical correction, should be the subject of further study.

Acknowledgement

The authors wish to thank the postgraduate study fund, Center of Excellence Fund, Chulalongkorn University, and the Thailand Research Fund, Senior Research Scholar for supporting the research and the measuring of nitric oxide related compounds. We would also like to thank Venerable Dr. Mettanando Bhikkhu of The Foundation of King Rama IX, the Great for reviewing the manuscript.

References

1. Hampl V, Herget J. Role of nitric oxide in the pathogenesis of chronic pulmonary hypertension. Physiol Rev 2000; 80: 1337-72.
2. Neidecker J. Pulmonary hypertension: the role of nitric oxide in adults. Rev Esp Anestesiol Reanim 2001; 48: 457-9.
3. Liaudet L, Soriano FG, Szabo C. Biology of nitric oxide signaling. Crit Care Med 2000; 28: N37-N52.
4. Takaya J, Teraguchi M, Nogi S, Ikemoto Y, Kobayashi Y. Relation between plasma nitrate and mean pulmonary arterial pressure in ventricular septal defect. Arch Dis Child 1998; 79: 498-501.
5. Narin F, Narin N, Pasaoglu H, Uzum K, Erdogan R. Nitric oxide level in children with pulmonary hypertension. Indian J Physiol Pharmacol 2000; 44: 335-9.
6. Takaya J, Ikemoto Y, Teraguchi M, Nogi S, Kobayashi Y. Plasma nitric oxide products correlate with cardiac index of congenital heart disease. Pediatr Cardiol 2000; 21: 378-81.
7. Jiang H, Zhang R, Gong H, Ma Z, Li X, Song H. Levels of plasma endothelin, nitric oxide and atrial natriuretic peptide after cardiopulmonary bypass and influence of inhaled nitric oxide in patients with ventricular septal defect and pulmonary hypertension. Zhonghua Wai Ke Za Zhi 2002; 40: 290-3.
8. Kotake F, Kobayashi J, Sonoda M, Komoda T. Nitric oxide-related compounds in patients with congenital heart defects and pulmonary hypertension. Pediatr Int 2000; 42: 249-54.
9. Ikemoto Y, Teraguchi M, Kobayashi Y. Plasma levels of nitrate in congenital heart disease: comparison with healthy children. Pediatr Cardiol 2002; 23: 132-6.
10. Lu H, Chen S, Wang H, Cheng P. Role of adrenomedullin in congenital heart disease associated with pulmonary hypertension. J Huazhong Univ Sci Technolog Med Sci 2003; 23: 275-7.
11. Ikemoto Y, Teraguchi M, Takaya J, Nogi S, Kobayashi Y. Plasma levels of nitric oxide products and endothelin in pulmonary hypertension with congenital heart disease. Acta Paediatr 1998; 87: 715-6.
12. Bishop JE, Guerreiro D, Laurent GJ. Changes in the composition and metabolism of arterial collagens during the development of pulmonary hypertension in rabbits. Am Rev Respir Dis 1990; 141: 450-5.
13. Moraes DL, Colucci WS, Givertz MM. Secondary pulmonary hypertension in chronic heart failure: the role of the endothelium in pathophysiology and management. Circulation 2000; 102: 1718-23.
14. Ozerol IH, Pac FA, Ozerol E, Ege E, Yologlu S, Temel I, et al. Plasma endothelin-1, homocysteine and serum nitric oxide values in patients with left-to-right shunt. Indian Heart J 2004; 56: 653-7.
15. Lam CF, Peterson TE, Croatt AJ, Nath KA, Katusic ZS. Functional adaptation and remodeling of pulmonary artery in flow-induced pulmonary hypertension. Am J Physiol Heart Circ Physiol 2005; 289: H2334-H2341.
16. Steinhorn RH, Russell JA, Lakshminrusimha S, Gugino SF, Black SM, Fineman JR. Altered endothelium-dependent relaxations in lambs with high pulmonary blood flow and pulmonary hypertension. Am J Physiol Heart Circ Physiol 2001; 280: H311-H317.
17. Levy M, Danel C, Laval AM, Leca F, Vouhe PR, Israel-Biet D. Nitric oxide synthase expression by pulmonary arteries: a predictive marker of Fontan procedure outcome? J Thorac Cardiovasc Surg 2003; 125: 1083-90.
18. Black SM, Bekker JM, McMullan DM, Parry AJ, Ovadia B, Reinhartz O, et al. Alterations in nitric oxide production in 8-week-old lambs with increased pulmonary blood flow. Pediatr Res 2002; 52: 233-44.

ระดับพลาสมาไนทริกออกไซด์ในผู้ป่วยเด็กที่มีภาวะหัวใจพิการแต่กำเนิดชนิดที่มีการไหลเวียนโลหิตไปปอดเพิ่มขึ้น

พัชร เกียรติสารพิภพ, พรเทพ เลิศทรัพย์เจริญ, ไพโรจน์ โชติวิทยธารากร, ยง ภู่วรวรรณ

ภาวะความดันโลหิตของหลอดเลือดที่ปอดสูงเป็นภาวะแทรกซ้อนที่สามารถพบได้บ่อยในผู้ป่วยเด็กที่มีโรคหัวใจพิการแต่กำเนิด ไนทริกออกไซด์เป็นสารที่หลั่งจากหลอดเลือดและมีความสำคัญต่อการขยายตัวของหลอดเลือด การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของความดันโลหิตที่ปอดต่อระดับพลาสมาไนทริกออกไซด์ในผู้ป่วยเด็กที่มีภาวะหัวใจพิการแต่กำเนิดชนิดที่มีการไหลเวียนโลหิตไปปอดเพิ่มขึ้น โดยทำการศึกษาระดับพลาสมาไนทริกออกไซด์ในผู้ป่วยเด็กที่มีภาวะหัวใจพิการแต่กำเนิดจำนวน 28 รายอายุตั้งแต่ 3 เดือนถึง 12 ปี ที่มีโรคหัวใจพิการแต่กำเนิดชนิดที่มีการไหลเวียนโลหิตไปปอดเพิ่มขึ้น โดยจำแนกผู้ป่วยออกเป็น 2 กลุ่ม คือกลุ่มที่มีโรคหัวใจพิการแต่กำเนิดชนิด left-to-right shunt และกลุ่มที่มีโรคหัวใจพิการแต่กำเนิดชนิด right-to-left shunt ทำการเก็บตัวอย่างเลือด ณ ตำแหน่งต่าง ๆ ของหัวใจจากการสวนหัวใจและทำการวิเคราะห์หาระดับพลาสมาไนทริกออกไซด์และไนไตรทโดยอาศัย Griess reaction พบว่าระดับพลาสมาไนทริกออกไซด์ในผู้ป่วยกลุ่มที่ 1 และกลุ่มที่ 2 ไม่มีความแตกต่างกันอย่างมีนัยสำคัญ และพบว่าในผู้ป่วยที่มีระดับความดันโลหิตที่ปอดสูงปานกลาง ระดับพลาสมาไนทริกออกไซด์มีความสัมพันธ์กับการเพิ่มขึ้นของความดันโลหิตที่ปอด ($r = 0.67$, p-value < 0.05) ผู้ป่วย 3 รายมีความดันโลหิตที่ปอดสูงมาก (mean pulmonary arterial pressure > 60) มีระดับพลาสมาไนทริกออกไซด์ลดลง จากการศึกษานี้อาจสรุปได้ว่าไนทริกออกไซด์มีส่วนสำคัญต่อการเปลี่ยนแปลงของความดันโลหิตที่ปอดในผู้ป่วยที่มีโรคหัวใจพิการแต่กำเนิดชนิดที่มีการไหลเวียนโลหิตไปปอดเพิ่มขึ้น