JECT. 2001;33:23–26
The Journal of The American Society of ExtraCorporeal Technology

The Influences of NO and Ach on cGMP Levels in Two Patient Populations

Zhen Jiang, MD;* Ye Lei,† MD; Kefang Gu, MD;* Jin Xianghua, MD;* Xia Liming, MD;† Hu Kejian,† CCP

**Department of Anesthesiology, †Department of Cardiac Surgery, Zhongshan Hospital, Medical College of Fudan University, Shanghai Cardiovascular Diseases Institute, Shanghai, China*

Abstract: Pulmonary hypertension following cardiac surgery is an important factor affecting postoperative mortality, and its mechanism has not been thoroughly clarified. Cardiopulmonary bypass (CPB) can destroy pulmonary endothelium and aggravate pulmonary hypertension. This study is designed to investigate the impacts of CPB on vascular endothelium-dependent relaxation, and the relations of CPB to pulmonary hypertension. Forty patients undergoing valve surgery were involved. According to their preoperative pulmonary arterial pressure (PAP), these patients were divided into pulmonary hypertension group (H group) and normal group (N group). The concentrations of cyclic guanosine monophosphate (cGMP) were measured at baseline conditions, after acetylcholine (Ach) injection, and during nitric oxide (NO) inhalation. Samples were taken before sternotomy and after weaning from CPB, 4 and 12 hours post-CPB. At baseline, the level of cGMP in the H group was lower than that of the N group by 33.9% before CPB. After initiating the CPB, although the level of cGMP continuously decreased in both groups until weaning from CPB (the N group decreased 33.3%, and the H group decreased 59%). At that point cGMP was higher in N group than in the H group ($p < .01$). The level of cGMP of both groups tended to recover 4 hours after CPB, but only the N group returned to baseline 12 hours after CPB. After injection of Ach, the level of cGMP of both groups followed the same change as in the baseline, except with different numeric value. The level of cGMP in N group rose ranging from 160.0–197.3%, while it rose ranging from 87.7–168.1% in H group. The levels of cGMP were higher in N group than those in H group at all times following injection of Ach (61.4, 173.3, 202.7, and 188.0%)($p < .01$). After inhalation of NO, the level of cGMP of both groups followed the same change as the baseline. The level of cGMP in N group rose ranging from 194.8–320.5%. Although the levels of cGMP were higher in N group than those in H group (6.9, 25.3, 23.3, and 16.6%), significant differences were achieved at the 4 and 12 hour post-CPB periods ($p < .05$ or $p < .01$, respectively). It was concluded that the injury of vascular endothelial function caused by CPB was more critical in pulmonary hypertension patients. **Keywords:** NO, acetylcholine cGMP, cardiopulmonary bypass. *JECT. 2001;33:23–26*

In patients with cardiac valvular diseases who present with pulmonary hypertension, the pulmonary function of endothelium-dependent vasodilation is often decreased (1). Cardiopulmonary bypass (CPB) has been shown to have the pulmonary endothelium (2). Pulmonary hypertension followed by cardiac surgery is an important causative factor in high postoperative mortality (3). Nitric oxide (NO), the main factor regulating vasomotion (4) and pulmonary hypertension, is thought to diffuse from the endothelium to adjacent muscle, and produce relaxation by activating guanylate cyclase and increasing cyclic guanosine monophosphate (cGMP) (5). Therefore, we investigated the impacts of pulmonary hypertension on the function of endothelium-dependent vasodialation and the impacts of CPB on nonendothelium-dependent vasodialation in patients with rheumatic heart diseases.

Address correspondence to: Dr. Zhen Jiang, Prof. of Anesthesiology, Vice-chairman of the Anesthesiology Department, Shanghai Cardiovascular Diseases Institute, 180 Fenling Road, Shanghai, 200032, China.
Received 15 August 2000; revised 10 October 2000.

MATERIALS AND METHODS

This study design was approved by the Ethics Committee of Zhongshan Hospital, Shanghai, China, and informed consent was obtained from all patients.

Forty patients in NYHA III classification undergoing mitral and aortic valve replacement were selected (sex: 24 males and 16 females; age: 36~62 years; weight: 47~72 Kg). According to preoperative assessment of pulmonary systolic pressure by Doppler echocardiography, these patients were divided into two groups: N group (with normal pulmonary pressure, $N = 22$) and H group (with pulmonary hypertension, $N = 18$ and PAP $= 57.8 \pm 14.0$ mmHg). Surgical procedures were performed using standard

general anesthesia. CPB with moderate hypothermia and moderate hemodilution was employed. Cardioplegia was given through the aortic root or by left and right sinuses of the coronary arteries. Blood samples were drawn from the radial artery before sternotomy, at weaning from CPB, and 4 and 12 hours after CPB. Concentrations of cGMP were measured at the basic conditions, after injection of Ach and during NO inhalation.

Application of Ach and NO

Ach 0.75 mg/mL was injected via internal jugalar vein, and blood samples were taken 10 min later. After an interval of 20 min, NO was administered at a concentration of 20 ppm, and blood samples were taken 5 min later. All blood samples were analyzed by I^{125}-cGMP radioimmunoassay to measure the concentration of cGMP (6).

Statistical Analysis

All values are presented as mean ± standard error of the mean. Differences between the two groups were analyzed by two-way analysis of variance (ANOVA). Statistical significance was accepted as the $p \leq .05$ level.

RESULTS

Forty patients undergoing valve replacement were studied. CPB and aortic clamp times were 87 ± 12 min and 58 ± 9 min, respectively. All patients were weaned from CPB successfully.

At baseline, the level of cGMP in the H group before CPB was lower than that of the N group by 33.9%. At the onset of CPB, cGMP decreased in both groups until weaning from CPB (the N group decreased 33.3%, while the H group decreased 59%), ($p < .01$). The level of cGMP of both groups tended recovered 4 hours after CPB, but only the N group returned to baseline levels (Fig. 1).

After injection of Ach, the level of cGMP in both groups followed similar changes. The level of cGMP in N group rose from 160.0–197.3%, while it rose from 87.7–168.1% in H group. The levels of cGMP were higher in N group than those in H group at all times after the injection of Ach (61.4, 173.3, 202.7% and 188.0%) ($p < .01$) (Fig. 2).

After inhalation of NO, the level of cGMP of both groups followed the same change as seen from baseline. The level of cGMP in N group rose from 194.8–320.5%, while the H group increased from 317.5–727.1% in H group.

DISCUSSION

The mechanism of pulmonary hypertension has not been thoroughly elucidated and is usually related to the decreased release of NO by the endothelium, and through elevated activity of vasoconstrictors such as endothelin (7). It has been shown that NO is the main factor regulating vasomotion (4), and its concentration is closely related to the generation of pulmonary hypertension (8). The process of CPB may instigate pulmonary hypertension through one, or more of the following conditions: the formation of embolism in microvasculature, leukocyte adherence to the endothelium, and the increase of thromboxane. All of these can destroy the pulmonary endothelium and aggravate pulmonary hypertension (2).

Because it is difficult to measure the concentration of NO in the body, we measured the concentration of cGMP, which can reflect the concentration of NO (5). The combination of Ach and M receptors can induce the endothelium to release NO (9), which indicates that it is endothelium-dependent vasodilation. By comparing the rising range between Ach injection and nitric oxide inhalation at the same timepoint, it was found that the level of cGMP rose more significantly in the group inhaling NO than that in the group of Ach injection. These findings indicate the endothelium-dependent vasodilation caused by Ach is

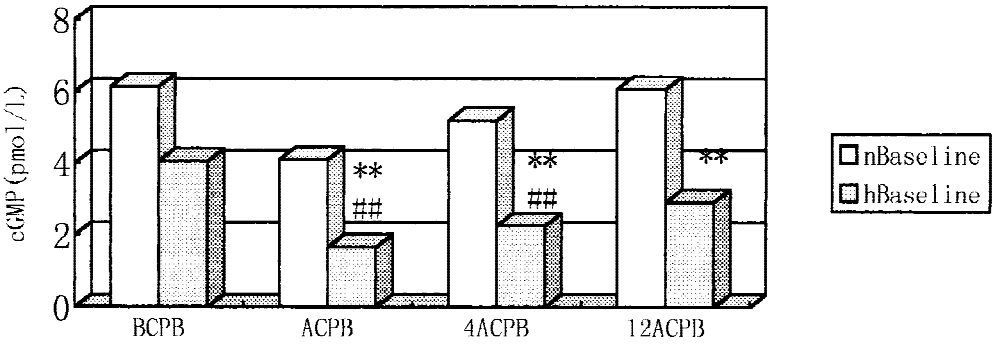

Figure 1. cGMP (pmol/mL) values.
ACPB: after cardiopulmonary bypass; 4ACPB: 4 hours after cardiopulmonary bypass; 12 ACPB: 12 hours after cardiopulmonary bypass; BCPB: before cardiopulmonary bypass; nBaseline: baseline of N group; hBaseline: baseline of H group.
Compared with BCPB: ## $p < .01$.
Compared with N group: ** $p < .01$.

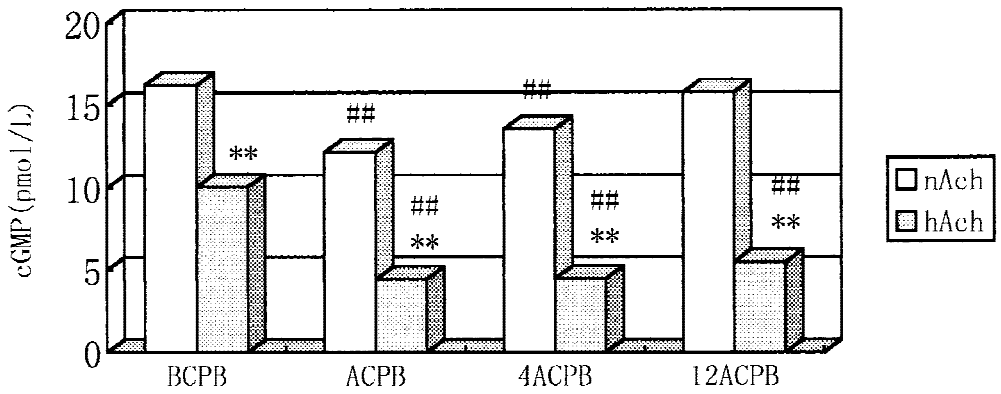

Figure 2. cGMP (pmol/mL) values at different times in two groups.
ACPB: after cardiopulmonary bypass; 4ACPB: 4 hours after cardiopulmonary bypass; 12ACPB: 12 hours after cardiopulmonary bypass; nACh: Ach injection of N group; hAch: Ach injection of H group; BCPB: before cardiopulmonary bypass.
Compared with BCPB: ## $p < .01$.
Compared with N group: ** $p < .01$.

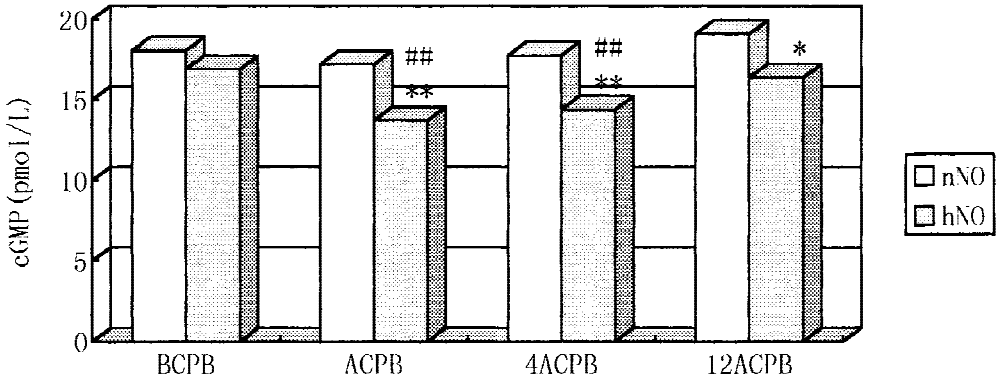

Figure 3. cGMP (pmol/mL) values at different times in two groups.
ACPB: after cardiopulmonary bypass; 4ACPB: 4 hours after cardiopulmonary bypass; 12ACPB: 12 hours after cardiopulmonary bypass; BCPB: before cardiopulmonary bypass; nNO: NO inhalation of N group; hNO: NO inhalation of H group.
Compared with before CPB: ## $p < .01$.
Compared with N group: * $p < .05$, ** $p < .01$.

weaker than that caused by nitric oxide inhalation, and also shows that endothelial function is abnormal.

Inhalation of 20 ppm NO significantly increased cGMP level in both groups. Before CPB, it elevated cGMP level by 195 and 318% in N group and H group, respectively. After CPB, the inhalation of NO also increased cGMP level. This effect was more obvious in N group. The cGMP level increased after CPB, recovering 12 hours after CPB in N group. The effect of inhalation of NO on cGMP level is more significant than that of Ach.

In the pulmonary hypertension group, the baseline cGMP dropped significantly after CPB and rose 12 hours later. However, after Ach injection and NO inhalation before CPB, cGMP rose 2.5 and 4 times of the baseline, respectively. Using NO inhalation after CPB, the cGMP of H group rose to 13.73 pmol/mL. Although cGMP was higher than baseline, it was still only 80% of maximum values. This showed that CPB impacts nonendothelium-dependent vasodilation by other pathways. Seccombe et al.'s (10) research indicates that G protein was responsible for the abnormality of endothelium-dependent vasodilation.

Comparing the cGMP of the two groups, we found that the baseline of cGMP in the N group was higher than that of the H group, and cGMP decreased less than that of the H group after CPB at 4 and 12 hours post-CPB. This indicates that endothelium-dependent vasodilation function of the N group was hindered less than that of the H group, and that the configuration and function of pulmonary endothelium of the N group was less abnormal than that of the H group. It can be concluded that the endothelial function of patients with pulmonary hypertension caused by heart disease is abnormal when compared with that in patients with normal pulmonary arterial pressure. The endothelial function will be more seriously effected by CPB in patients with pulmonary hypertension.

ACKNOWLEDGMENT

This study was supported by the Ministry of Public Health of China.

REFERENCES

1. Loscalzo J. Endothelial dysfunction in pulmonary hypertension. N Engl Med. 1992;327:17–20.
2. Koul B, Wollmer P, Willen H, et al. Venoarterial extracorporeal membrane oxygenation—How safe is it? J Thorac Cardiovsc Surg. 1992;104:579–84.
3. Wheller J, George B, Muller D, et al. Diagnosis and management of postoperative pulmonary hypertensive crisis. Circulation. 1980;60: 1640–9.
4. Stamler JS, Loh E, Roddy MA. Nitric oxide regular basal systemic and pulmonary vascular resistance in healthy humans. Circulation. 1994;89:2035–40.
5. Ignarro LJ, Harbison RG, Wood KS, et al. Activationon of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: Stimulation by acetylcholine, bryadykinin and arachidonic acid. J Pharmacol Exp Therap. 1986; 237:893–900.
6. Journois D, Poward P, Mauriat P. et al. Inhaled nitric oxide as a therapy for pulmonary hypertension after operations for congenital heart diseases. J Thorac Cardiovasc Surg. 1994;107:1129–35.
7. Abman S, Chatfield B, Hall S, et al. Role of EDRF during transition of pulmonary circulation at birth. Am J Physiol. 1990;259:H1921–7.
8. Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. Circ Res. 1990;66:1561–75.
9. Vane JR, Aanggad EE, Botting RM. Regulatory function of vascular endothelium. N Engl J Med. 1990;323:27–36.
10. Seccombe JF, Pearson PJ, Schaff HV, et al. Oxygen radical mediated vascular injury selectively inhibits receptor-dependent release of nitric oxide from canine coronary arteries. J Thorac Cardiovasc Surg. 1994;107:505–9.