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Effects of L-Arginine Cardioplegia on Myocardium

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Abstract: Infusion of L-arginine (a precursor of nitric oxide, NO) in cardioplegia was examined to test its effect on metabolism of myocardium after myocardial ischemia and reperfusion (IR). Twenty-eight patients undergoing valve replacement were involved and randomly divided into two groups: the control group (crystalloid cardioplegia) and the experimental group (crystalloid cardioplegia + L-arginine). Blood samples were taken both before aortic clamping and after aortic unclamping from right radial artery to measure the concentrations of NO_2^-/NO_3^-, lactic acid (LA), malondialdehyde (MDA), superoxide dismutase (SOD), and xanthine oxidase (XOD). In the control group, the NO_2^-/NO_3^- level decreased at aortic unclamping, and 30 min later, it decreased significantly as compared with that before aortic clamping ($p < .05$). In the experimental group, it increased at aortic unclamping ($p < .05$), and 60 min later, increased to the peak. Five, fifteen, and thirty min after aortic unclamping, the concentrations of LA and MDA in the experimental group were lower than those in the control group ($p < .05$). Thirty and sixty min after aortic unclamping, the concentrations of SOD remained higher in the experimental group than those in the control group ($p < .05$). There was no difference between groups in the concentrations of XOD. The addition of L-arginine to the cardioplegia can protect the myocardium from injury by releasing nitric oxide. **Keywords:** free oxygen radical, L-arginine, ischemia/reperfusion. *JECT. 2001;33:10–14*

Myocardial ischemia followed by reperfusion (IR), injuries the coronary artery endothelium, and inhibits the synthesis of nitric oxide (NO) (1). NO acts as an endogenous vasorelaxator (2) and an inhibitor of platelet aggregation (3). It also exerts an antineutrophil adherence effect to vascular endothelium (4). It has been shown that L-arginine (L-arg), the precursor of NO, can increase the endogenous NO by the L-arginine–NO pathway (5). Therefore, the purpose of this study was to examine L-arginine cardioplegia to test its impact on myocardial metabolism after IR.

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.

This study was performed in 28 patients undergoing aortic and mitral valves replacement operations. The patients were randomly divided into two groups: the control group and the experimental group. The demographic data of the two groups were shown in Table 1.

The 28 patients had general anesthesia. Cardiopulmonary bypass (CPB) using centrifugal pump and membrane oxygenation (Maxima, Medtronic Cardiopulmonary, Minneapolis, MN). The priming volume was 30 mL/kg weight. Activated clotting time (ACT) was kept above 480 sec. The flow rate was kept between 50–70 mL/kg/min. The temperature of nasopharynx was kept between 27–29°C. The crystalloid cardioplegia or L-arginine cardioplegia (5 g L-arg per 1 Liter crystalloid cardioplegia) at 4°C was administered by the left and right sinuses of coronary artery 20 mL/kg for the loading dose and then every 30 min at 10 mL/kg.

Before aortic clamping and 5, 15, 30, 60, and 120 min after aortic unclamping, blood samples from radial artery were taken to measure the concentrations of lactic acid (LA), malondialdehyde (MDA), superoxide dismutase (SOD), and xanthine oxidase (XOD). NO concentration was determined by reconverting its oxidation end-products (nitrite, NO_2^-, and nitrate, NO_3^-). Before aortic clamping and 5, 30 min 1, 2, 24, and 48 hours after aortic unclamping, the blood samples from radial artery were taken to measure the concentrations of NO_2^-/NO_3^-. All the samples were tested by a UV-754 ultraviolet spectrophotometer.

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Table 1. General Materials of Patients in Two Groups.

	Control group	Experimental group
Cases	7	7
Age (y)	45.0 ± 9.8	45.6 ± 7.2
Sex (M/F)	3/4	2/5
Weight (Kg)	57.0 ± 10.0	60.1 ± 7.7
Heart Function	III-4, IV-3	III-4, IV-3
EF (%)	30.7 ± 5.0	35.6 ± 5.0
T-CPB (min)	96.3 ± 6.0	90.2 ± 14.6
T-AC (min)	67.6 ± 8.7	59.3 ± 14.2

EF: ejection fraction.
T-AC: time of aortic clamping.
T-CPB: time of cardiopulmonary bypass.

Statistical Analysis

All data are given as mean plus or minus standard error of the mean. Differences between the two groups were analyzed by two-way analysis of variance (ANOVA). The level of significance was established at $p < .05$.

RESULTS

Table 1 shows that before operation, there were no differences of clinical variables between the two groups. There were also no differences in time of cardiopulmonary bypass and aortic clamping

Concentration of NO_2^-/NO_3^-

After aortic unclamping, the concentration of NO_2^-/NO_3^- in control group dropped and reached a nadir after 60 minutes (decreased 21.5%); while in the experimental group it rose and arrived to peak 60 min later (80.9%). Significant differences between groups were evident ($p < .05$) (Fig. 1).

Concentration of LA

After aortic unclamping for 5 min, LA rose 53.5% more than that of before aortic clamping in the control group ($p < .05$); while in the experimental group, LA rose significantly 30 min later ($p < .05$). The concentration of LA in the experimental group was lower than that of the control group from 5 to 30 min after aortic unclamping ($p < .05$) (Fig. 2).

Concentration of MDA

After aortic unclamping, MDA rose 42.8% compared to pre-aortic clamping in the control group ($p < .05$); while in the experimental group, MDA rose significantly 15 min later ($p < .05$). The concentration of MDA in the experimental group was lower than that of the control group at any time after aortic unclamping ($p < .05$) (Fig. 3).

Concentration of SOD

After aortic unclamping, the concentrations of SOD of both groups dropped 18.3 and 14.6%, respectively, at 15 min. However, SOD in the experimental group was maintained higher than that in the control group at 30 and 60 min ($p < .05$) (Fig. 4).

Concentration of XOD

After aortic unclamping, though the concentrations of XOD of both groups rose, there were no significant differences between the two groups at any time. (Fig. 5).

DISCUSSION

Studies have shown that both regional and global ischemia and reperfusion injure the endothelium (6). The endothelial dysfunction may be expressed as a reduced production or release of endothelium-derived relaxing factor—NO (7). Tests have been made to determine if the endogenous L-arginine-NO pathway is involved in myocardial injury. The application of L-arginine may be an easier and more convenient method to use.

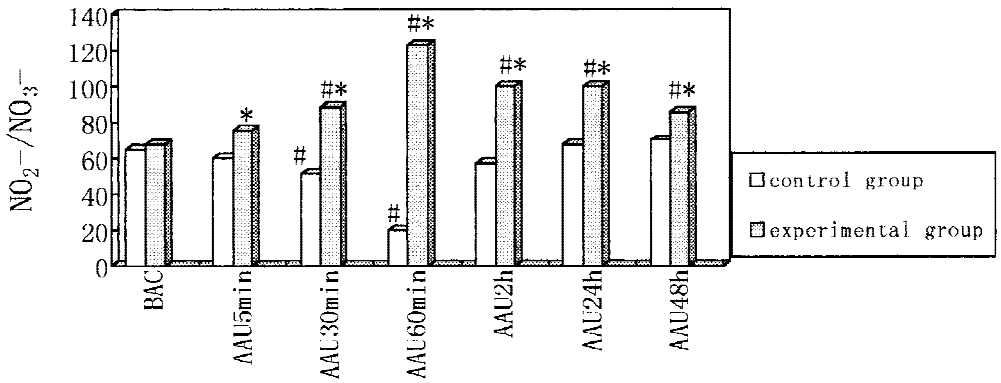

Figure 1. Changes of $NO_2^-/_3^-$ of both groups. AAU: after aortic unclamping; BAC: before aortic clamping.
Comparison between the groups: *$p < .05$.
Comparison between BAC and AAU: #$p < .05$.

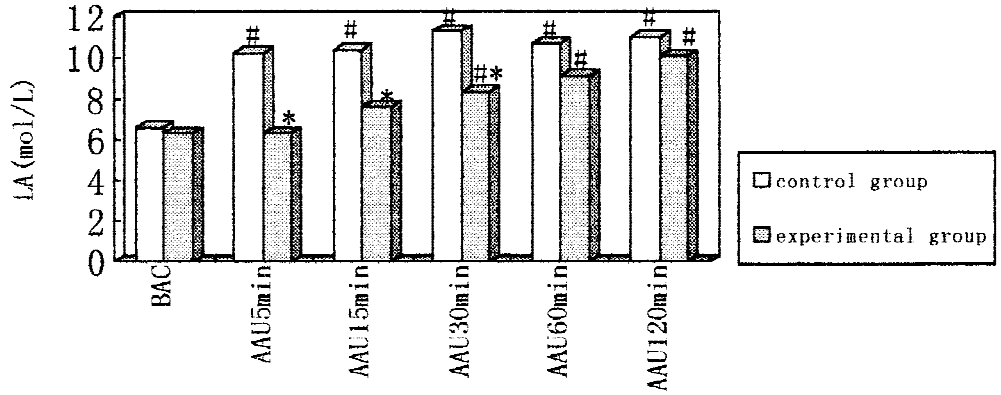

Figure 2. Changes of lactic acid (mol/L) of both groups. AAU: after aortic unclamping; BAC: before aortic clamping.
Comparison between the groups: *$p < .05$.
Comparison between BAC and AAU: #$p < .05$.

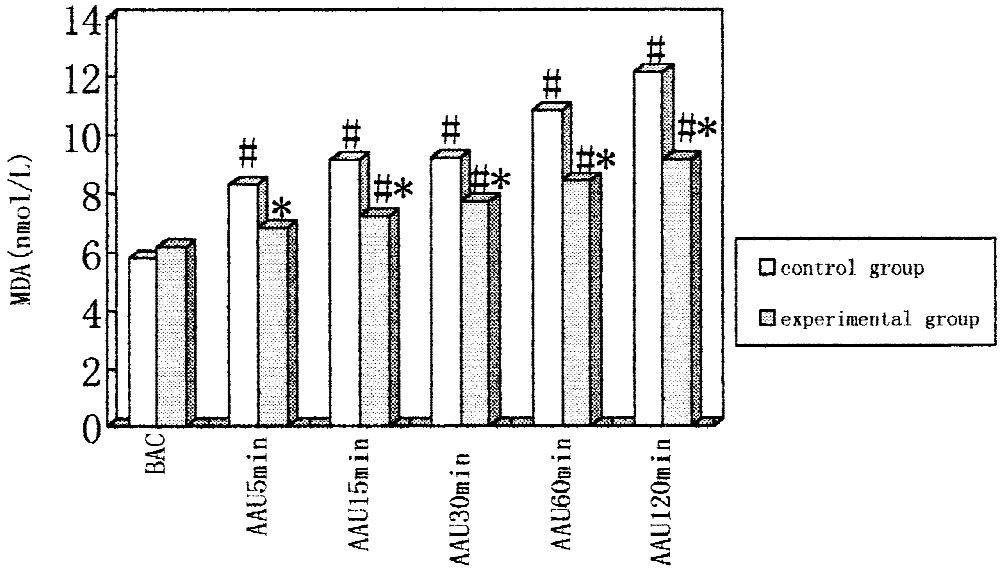

Figure 3. Changes of MDA (nmol/L) of both groups. AAU: after aortic unclamping; BAC: before aortic clamping.
Comparison between the groups: *$p < .05$.
Comparison between BAC and AAU: #$p < .05$.

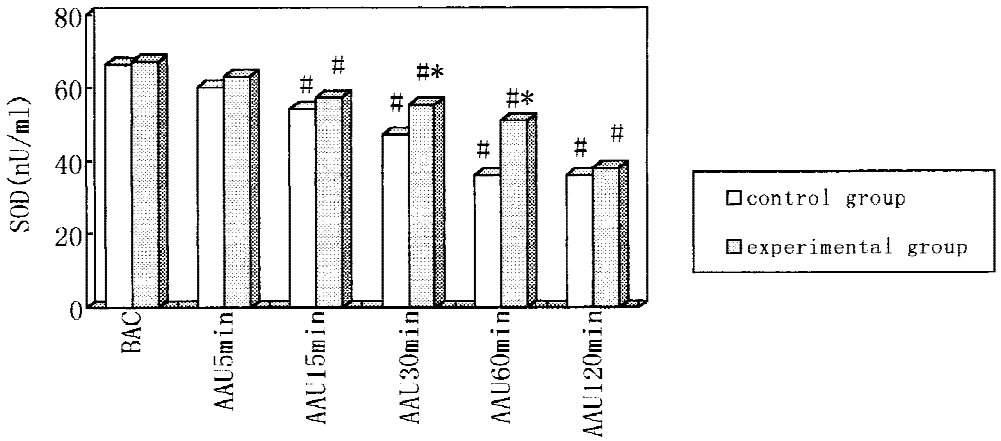

Figure 4. Changes of SOD (nU/mL) of both groups. AAU: after aortic unclamping; BAC: before aortic clamping.
Comparison between the groups: *$p < .05$.
Comparison between BAC and AAU: #$p < .05$.

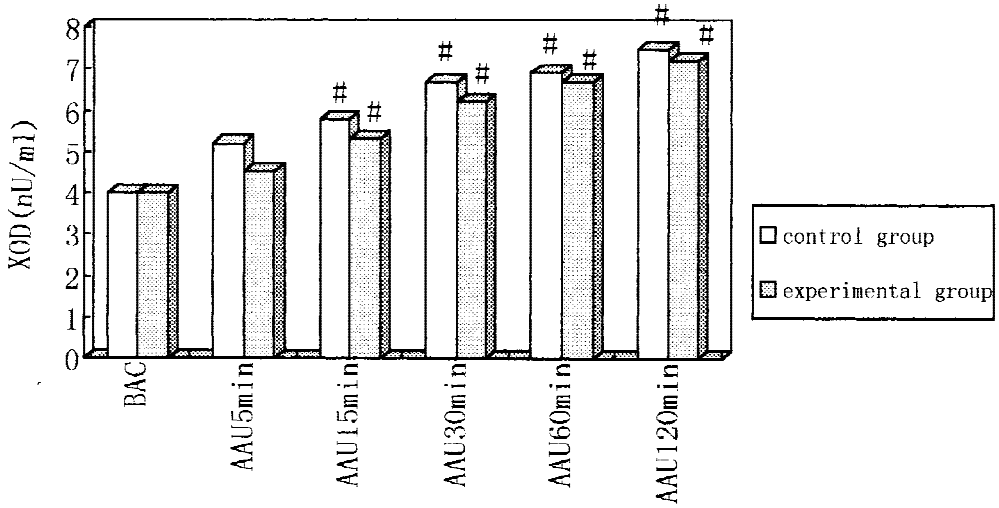

Figure 5. Concentration changes of XOD (nU/mL) of both groups. AAU: after aortic unclamping; BAC: before aortic clamping.
Comparison between groups> *$p < .05$.
Comparison between BAC and AAU: #$p < .05$.

These data showed that after aortic unclamping, the concentrations of NO_2^-/NO_3^- dropped to nadir at 60 min in the control group, indicating that synthesis of NO by endothelium was injured by IR (1, 6); while NO rose to peak at 60 min in the experimental group, indicating that by L-arginine–NO pathway, the synthesis and release of NO increased. The quantity of NO_2^-/NO_3^- in the experimental group was much higher than that in the control group, showing that by L-arginine–NO pathway, the nitric oxide synthase (NOS) of endothelium was strengthened, and the synthesis of NO increased (8). In the early stage, the increase of NO can increase the flow of coronary flow, thus promoting the recovery of myocardium after IR. It was found that NO_2^-/NO_3^- was elevated 24 hours later, possibly due to the activation of induced NOS (iNOS) (9), which can be released independent of Ca^{2+}. However, in the control group, the endothelial dysfunction may be responsible for the decreased release of NO after CPB (6, 10).

It was noted that 5 to 30 min after aortic unclamping, LA in the experimental group was lower than that in the control group. This may be related to the level of ATP in the cells. We think that NO may function to decrease the decomposition of ATP or promote the composition of ATP, which needs further research. MDA results from the membrane of myocardium reacting with oxygen free radical (OFR). It was lower in the experimental group than that in the control group. This indicates that L-arg may have direct protective effects on the cellular membrane or the formation of NO inhibit the activities of neutrophil (4, 10). During IR, the production of OFR is mediated mainly by neutrophil and XOD. However, there was no difference in the activity of XOD in both groups at any time. Therefore, it can be explained that L-arginine reduced neutrophil accumulation on cellular membrane by attaching to membrane or the enhanced NO production protects endothelium through antineutrophil and quenching superoxide. SOD was higher in the experimental group during aortic cross clamping, indicating that NO could retain the activity of SOD by consuming OFR. Furthermore, the antineutrophil adherence of NO to vascular endothelium actions decreases neutrophil adherence and, thus, lowers the regional production of OFR. Therefore, by the L-arginine–NO pathway, endothelial production of NO is strenghened, which reverses the endothelial injury by neutrophil–mediated damage and quenching superoxide.

In conclusion, it is known that by the L-arginine–NO pathway, the synthesis of NO by endothelium is strengthened. L-arg is converted into NO, which can relieve or reduce IR injury: it may be used as an energy source during ischemia, as an drug to decrease neutrophil's adherence, and to reduce production of OFR. Therefore, inclusion of L-arginine in cardioplegic solution can be a safe and low-cost adjuvant.

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