Impaired Endothelial Responses in Patients with Deep Hypothermic Cardiopulmonary Bypass

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Summary: We examined whether vascular endothelial function is impaired with deep hypothermia during cardiopulmonary bypass. Such impairment may cause the prolonged hypoperfusion of tissues or organs. Thirty adult patients were classified into three groups according to the degree of hypothermia during cardiopulmonary bypass; deep (DH: 18-20°C, n=10), moderate (MDH: 25-30°C, n=10) and mild (MLH: 32-34°C, n=10) hypothermia. A bolus dose of 100 mg acetylcholine was injected, followed by 1 mg nitroglycerine 20 min later under hypothermic cardiopulmonary bypass. Plasma concentrations of nitric oxide metabolites (NOx) and endothelin-1 were determined until the 7th post-operative day (POD). The reduction of arterial pressure after acetylcholine was the smallest in the DH group (-9±6 mmHg, p<0.01). The time to reach the maximum reduction of arterial pressure was the longest in the DH group (acetylcholine: 47.7±23.6 sec, nitroglycerine: 53.4±21.4 sec, p<0.01). NOx production was reduced until the 1st POD and then recovered. The recovery was more rapid in the MLH group than the other two groups (p<0.01). The plasma endothelin-1 did not differ among the three groups. These results suggest that endothelial function is impaired during cardiopulmonary bypass with deep hypothermia.

Key words acetylcholine stress test, deep hypothermia, nitric oxide, endothelin

INTRODUCTION

Hypothermia has been employed to promote ischemic tolerance during surgery reducing tissue basal oxygen consumption [1]. Currently, deep hypothermia combined with cardiopulmonary bypass (CPB) is a common technique for protecting the brain from ischemic injury during aortic arch surgery, in which temporary circulatory arrest is frequently unavoidable [2]. However, post-operative impairment of tissue or organ microcirculation may be an important consequence of hypothermic CPB [3,4]. Some patients with hypothermic CPB were reported to develop severe organ dysfunction, resulting in increased surgical morbidity and mortality [5]. Endothelial dysfunction has been highlighted as a possible cause of prolonged hypoperfusion and reperfusion injury after hypothermic CPB [6].

The vascular endothelium plays an important role in maintaining perfusion by regulating vasomotor tone. It is now clear that the endothelium modulates vascular tone by releasing endothelium-derived factors, such as nitric oxide (NO) and endothelin (ET-1). NO relaxes vascular smooth muscle, whereas ET-1 constricts it. Acethylcholine (ACh) acts indirectly by inducing the endothelial cells to make and release NO, which then signals the smooth muscle cells relax. This effect of NO on blood vessels provides an explanation for the mechanism of action of nitroglycerine (NG). NG is converted to NO, which relaxes blood vessels, thereby NG is recognized as a direct vascular smooth muscle relaxant. Therefore ACh is used clinically to evaluate the endothelium-dependent dilator response of the coronary artery.
Recently, deleterious effects on vascular responses have been shown after hypothermic CPB in lambs [7]. Increased NO or ET during hypothermic CPB may influence endothelial function [8,9]. Nevertheless, there has been no report showing adverse effects on vascular responses of hypothermic CPB in patients.

The purpose of the present study is to evaluate the endothelial responses during deep hypothermia by recording the responses to ACh and NG and the changes of the blood concentrations of NOx (nitrate and nitrite, the terminal metabolites of NO), and ET-1 at three different cooling levels during hypothermic CPB in patients.

**PATIENTS AND METHODS**

This study was approved by the Ethics Committee of Kurume University School of Medicine. Written informed consent was obtained from all the subjects before starting the study. Thirty patients undergoing cardiovascular surgery with hypothermic CPB were enrolled. Patients with complications from failure of vital organs, including the brain, lung, kidney or liver, or with metabolic insufficiencies, were excluded. The patients were in the fasting state for at least 12 hrs before surgery. They were divided into three groups according to the lowest rectal temperature (LRT) attained during CPB; mild hypothermia (MLH) group with LRT between 32 and 34°C (n=10), moderate hypothermia (MDH) group with LRT between 25 and 30°C (n=10), and deep hypothermia (DH) group with LRT below 20°C (n=10). Throughout the study the subjects received no other drugs which could release NO such as sodium nitroprusside. Nine additional patients undergoing cardiovascular surgery with CPB were selected for assessment of blood cholinesterase (Ch-E) activity. In these patients a 2 ml sample of arterial blood was withdrawn for Ch-E determination after induction. The subjects were premedicated with oral flunitrazepam (1 mg) 1 hr before going to the operating room. Anesthesia was induced with intravenous fentanyl (300 μg) and midazolam (10 mg) with the supplementary inhalation of sevoflurane (1-2% end-tidal). The trachea was intubated following intubation with the trachea of sevoflurane (1-2% end-tidal). The trachea was intubated following intubation with the trachea of sevoflurane (1-2% end-tidal). The trachea was intubated following intubation with the trACHE-E) with the surgical incision. Anesthesia was maintained with additional injections of fentanyl and sevoflurane inhalation (1-2% end-tidal) as needed. The rectal temperature was monitored throughout the study. The radial artery was cannulated for arterial blood pressure monitoring and blood sampling. A pulmonary artery (PA) catheter was introduced through the right internal jugular vein. During CPB, the blood flow was kept at 2.4 L min⁻¹ m⁻² and the perfusion pressure was adjusted to between 50 and 70 mmHg. Hemodynamic variables including mean arterial pressure (AP), central venous pressure (CVP), mean pulmonary artery pressure (PAP), and cardiac output (CO) were simultaneously recorded at the time of blood sampling. ACh (100 mg) was rapidly injected within seconds as a bolus dose through the CVP line in a period of stable rectal temperature and perfusion pressure under CPB with aorta cross clamping and constant flow. NG (1 mg) was injected in the same manner 20 min after ACh. Radial AP was continuously recorded for 20 min following each bolus injection. The maximum reduction of the mean radial AP (AP_{max}) was the maximum response to each drug. T_{max} was the time from the ACh or NG injection to AP_{max}. The time for recovery of the mean radial AP (T_{rec}) to the pre-injection level was also observed. The AP reduction rate was calculated as AP_{max} divided by T_{max}. ACh and NG were administered only once in each patient, when rectal temperature had reached its lowest level during CPB.

Samples of 5 ml of arterial blood for measuring the plasma concentration of NOx and ET-1 were withdrawn at the following times: after anesthesia induction (P1), during CPB before drugs injection (P2), after weaning from CPB (P3), at the end of surgery (P4), on the 1st post-operative day (POD1) (P5), POD3 (P6), POD7 (P7). The plasma concentration of NOx was measured using high performance liquid chromatography based on the Griess's reaction (ENO 10, EICOM, Japan), and the plasma concentration of ET-1 was obtained by ELISA (Immuno-Biological, Tokyo, Japan). Serum Ch-E activity was determined with the oxidase method on the blood sample at four different temperatures (20, 28, 34 and 37°C) in vitro.

The statistical significance of differences among the three groups was tested using the SPSS version 10.03 program. The sequential changes of the variables in each group were tested with a paired t-test. The plasma concentrations of NOx and ET-1 were compared among the three groups by using ANOVA and post hoc Bonferroni test. The test was used for post hoc comparison when analysis of variance was significant. Differences were considered significant at p<0.05. All data were presented as the mean±SD.
RESULTS

The patient characteristics in each group are shown in Table 1. There were no differences in the patient’s profiles among the three groups. LRT during hypothermic CPB was 33±1 in MLH, 29±1 in MDH and 19±2°C in DH.

Hemodynamic variables including mean AP, CVP, mean PAP, and CO did not differ among the three groups. The mean AP before ACh injection was maintained around 60 mmHg in each group (56±10 mmHg in DH, 60±7 in MDH and 60±10 in MLH). The mean AP dropped transiently after ACh injection in all of the three groups (p<0.05) (Fig. 1). APmax was the smallest in the DH group (-9.3±5.6 mmHg, p<0.01) compared with the other two groups (-20.0±7.5 mmHg in MDH and -19.0±7.5 in MLH). The following NG injection also decreased the mean AP in all of the groups (p<0.05) (Fig. 1), and the relative reduction of APmax after NG was the smallest in the DH group among the three groups (p<0.01) (-12.3±3.5 mmHg in DH, -24±5.4 in MDH and -22±7.5 in MLH). The APmax in the DH group was larger after the NG injection than after the ACh injection (p<0.05). Both Tmax and Trc after the ACh or NG injection were longer in the DH group than in the other two groups (p<0.05) (Table 2).

The plasma NOx concentrations after anesthesia induction were not different among the three groups (59±4 μM in DH, 51±2 μM in MDH and 54±4 μM in MLH). The plasma NOx concentrations decreased with cooling on CPB in all groups (p<0.05), while the reduction rate was independent of the degree of hypothermia (Fig. 2). The low level of plasma NOx concentration continued until POD1 in all the three groups. The plasma NOx concentration recovered to

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<th>TABLE 1. Patient characteristics and LRT during hypothermic CPB</th>
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<td>Age (Yr)</td>
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<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<th>TABLE 2. Tmax and Trec of AP after ACh and NG injections under hypothermic CPB</th>
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<td>Time (sec.)</td>
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<tr>
<td>Tmax-ACh</td>
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<td>Tmax-NG</td>
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<td>Trec-ACh</td>
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* p<0.05 vs MLH and MDH groups, † p<0.05 vs MLH group, ‡ p<0.05 vs Trec-NG in MDH group

Fig. 1. Relative changes of AP after ACh or NG injection under hypothermic CPB with constant flow. ●, DH group (n=10); ▲, MDH group (n=10); □, MLH group (n=10). All data are mean ± SD. * p<0.05 vs baseline, † p<0.05 vs MLH, MDH group, ‡ P<0.05 vs DH group after ACh.

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Fig. 2. Sequential NOx plasma concentrations. The measuring points (P1 through P7) are defined in the patient and methods section. All data are mean ± SD. * p<0.05 vs baseline, # p<0.05 vs MLH group

Fig. 3. Sequential ET-1 plasma concentrations. The measuring points (P1 through P7) are defined as in Fig. 2. All data are mean ± SD.

TABLE 3.
Serum Ch-E activity at four different blood temperatures

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<tr>
<th>Blood Temperature</th>
<th>Ch-E activity (L.U.L.⁻¹)</th>
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<tr>
<td>37 °C (n=9)</td>
<td>1400±250</td>
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<td>34 °C (n=9)</td>
<td>1600±250</td>
</tr>
<tr>
<td>28 °C (n=9)</td>
<td>950±100</td>
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<tr>
<td>20 °C (n=9)</td>
<td>400±70</td>
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Serum Ch-E activity was depressed with cooling. Serum Ch-E activity at 20 °C decreased to about 30% of the baseline activity at 37 °C (Table 3).
DISCUSSION

In this study, changes in mean AP were continuously recorded following ACh or NG injections during CPB. During CPB with constant flow and aorta clamping, the changes in mean AP reflected changes in the systemic vascular resistance of the whole body. Deep hypothermia with LRT of 19±2°C on CPB impaired both endothelium-dependent (ACh) and -independent (NG) vascular relaxation, the former more than the latter. Our findings suggest that both a direct inhibitory effect on the vascular smooth muscle and an impairment of the endothelial function are responsible for the blunting of the vascular responses in the DH group.

We used ACh to induce endothelium-dependent vascular relaxation and NG to achieve endothelium-independent vascular relaxation in this study. The injection dose causing 40% reduction in mean AP in the MLH on CPB was 100 μg ACh and 1mg NG. Weis M et al. [10] reported that a smaller dose of ACh than of NG (50 μg vs. 300 μg) was required to achieve maximum dilatation of the coronary artery, when the drug was injected directly into the coronary artery. These discrepancies in the dose of ACh and NG needed for a similar degree of vascular relaxation may be related to differences in the site of injection and the biological half-life of each drug. In our study, ACh and NG took a longer route to reach the vascular bed compared with direct injection into the coronary artery. We injected the drugs through a CVP catheter into the CPB circuit, and the drugs had to pass through the extracorporeal bypass route including an artificial membrane lung and a reservoir. Thus, the volume in which the drugs were diluted was much larger in our study than in study by Weis et al. [10]. In addition, hypothermia may decrease the drug potency. It is hard to determine the plasma concentrations of ACh precisely in clinical situations because of its short biological half-life. ACh is likely to have been more rapidly degraded into non-active metabolites than NG on arrival at the endothelium of the vascular bed, because NG has a relatively longer biological half life [11]. Even though such a high dose of ACh was used to cause the vascular response, we could find no evidence of vasoconstriction, which might be evoked by the direct action of ACh on the muscarinic receptor of vascular smooth muscle. ACh is metabolized into choline and acetic acid by serum Ch-E within several seconds. Our in vitro study showed that the serum Ch-E activity was inhibited by cooling of the blood to 28 and 20°C. The maximum inhibition was at 20°C. Since we injected the same dose of ACh in each group, the ACh concentration acting on the endothelial cells should have been the highest in the DH group. Thus, vascular relaxation was impaired in the DH group even though the injected dose of ACh might have produced a greater concentration at endothelial cells in the DH patients. The impairment of vascular relaxation did not depend on the pharmacokinetic changes of ACh, but on the vascular endothelium and smooth muscle at the response site.

The decrease in APmax with ACh injection was greater than that with NG in the DH group, while there was no difference in the drug-induced APmax reduction between ACh and NG in either the MLH or the MDH groups. It seems that endothelium-dependent vascular relaxation was more severely impaired than endothelium-independent vascular relaxation in deep hypothermia. ACh relaxes the vascular tone by producing NO through the muscarinic receptor on the vascular endothelium. The biological process of NO production by the constitutive endothelial NO synthase could be depressed in deep hypothermic CPB. Slowing the biological process in hypothermia includes the reaction of cyclic GMP in the vascular smooth muscle. The coupling of calcium to the contractile protein would be augmented in the vascular smooth muscle, due to slowing of calcium mobilization in the cytosol. Therefore, the speed of relaxation of the vascular smooth muscle would be impaired in hypothermic conditions [12], as shown in the present study.

NOx concentration, which decreased after the initiation of CPB until POD 1, returned to the baseline level at POD 3. Endothelial cells may resume NO production at POD 3. On the other hand, some reports have shown an elevation of the plasma NOx level during and after CPB [8,13]. Moreover, several inflammatory factors are reported to activate the expression of iNOS and NO production [14]. This complicated mechanism seems to exist in the pathogenesis of NO production induced after CPB. Further investigation is needed to clarify the relationship between the amount of NO production and the activation of the NO donor.

ET-1 is known as a powerful vasoconstrictor releases by the vascular endothelium. Many factors, such as shear stress, thrombin, and hormones have been shown to stimulate production ET-1 [15,16]. Knothe et al. [17] reported an elevation of the plasma ET-1 level after open heart surgery. In the present study, neither an increase in plasma ET-1 nor a dif-
ference of plasma ET-1 among the three groups was observed. The baseline value of AP also did not differ among the three groups under CPB which constant flow. Therefore, the vascular tone at the beginning of the ACh Stress test was considered to be similar among the three groups.

Vascular tone is modulated by the balance between vasodilating (NO) and vasoconstricting (ET) factors. In particular, CPB provokes a marked stress response, which has been quantified by measuring hormones and vasoactive substances in plasma [18,19]. Reves et al. [20] reported that the epinephrine level increased markedly throughout CPB, and Tokunaga et al. [21] found that plasma catecholamine level varied with temperature during CPB. Changes in catecholamine concentration might have affected the results of this study. Generally, α-adrenergic sympathetic nerves constrict thermoregulatory arteriovenous shunts with lowering body temperature. Autonomic activation may also have modulated the vascular tone during hypothermic CPB. However, changes in systemic hemodynamics are not prominent when the vasoconstriction is limited to thermoregulation because only about 10% of cardiac output traverses the microcirculation, and larger vessels such as arterioles controlling blood pressure are therefore rarely influenced by hypothemic condition. Nervous system function, including peripheral sympathetic activity, was heavily depressed when the body temperature decreased below 20°C.

While deep hypothermia combined with CPB is indispensable to protect the brain from ischemic injury during aortic arch surgery, impairment of the microcirculation is reported as one of the causes responsible for the prolonged dysfunction of vital organs, such as the liver, kidney or lung after deep hypothermic CPB [22,23]. Endothelium dysfunction developed during deep hypothermia on CPB seems to continue for several days after CPB, considering the prolonged reduction of NOx production. The vascular endothelium has been shown to play an important role in maintaining hemodynamics by modulating membrane permeability, lipid transport, vasomotor tone, coagulation and fibrinolysis [24]. The impairment of the vascular endothelium could be responsible for vasospasm, coagulopathy, and systemic inflammatory response [25].

In conclusion, our findings indicated that the blunting of the vascular response to ACh was more prominent than that to NG during deep hypothermic CPB. This suggests that deep hypothermic CPB causes more severe impairment of the endothelial function than of the smooth muscle function.

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Impaired Endothelial Response


