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Effect of Selective Antegrade Cerebral Perfusion with Moderately Hypothermic Lower Body Circulatory Arrest on Biomarkers Related to Endothelial Function

Antegrad Serebral Perfüzyon ve Distal Ilımlı Hipotermik Sirkülatuar Arrest Tekniğinin Endotel Fonksiyonuna İlişkin Biyobelirteçler Üzerine Etkisi

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ABSTRACT

Aim: This study aims to compare biomarkers related to endothelial function during selective antegrade cerebral perfusion with moderate hypothermic lower body circulatory arrest with that of standard cardiac surgery.

Material and Methods: Thirty-six consecutive patients who underwent selective antegrade cerebral perfusion with moderately hypothermic lower body circulatory arrest at 28°C (study group) for aneurysms of the ascending aorta were prospectively compared with 36 patients who underwent standard cardiac surgery (control group) with conventional cardiopulmonary bypass. Nitric oxide, asymmetric dimethylarginine, hydrogen sulfide and total antioxidant capacity status and lactate levels in blood specimens obtained from the vena cava inferior were studied. Clinical results and biochemical parameters were evaluated.

Results: Biomarkers related to endothelial function were found to be similar between the groups except for asymmetric dimethylarginine. The asymmetric dimethylarginine levels were lower, while lactate levels were significantly higher compared to the control group. When the patients with coronary artery disease were excluded from the analysis to rule out the predominance of coronary artery disease patients in one group as a confounding factor, the asymmetric dimethylarginine levels were found to be similar between the two subgroups.

Conclusion: Low plasma levels of asymmetric dimethylarginine in the study group may have a protective role in endothelial nitric oxide synthesis. When patients with coronary artery disease were excluded from both group, biomarkers related to endothelial function were similar in both groups. We consider that endothelial functions are not affected adversely during short periods of moderately hypothermic lower body circulatory arrest.

Key words: Nitric oxide, asymmetric dimethylarginine, hydrogen sulfide, thoracic surgery.

ÖZ

Amaç: Bu çalışma, antegrad serebral perfüzyon ve distal ılımlı hipotermik sirkülatuararrest tekniğinin endotel fonksiyonuna ilişkin biyobelirteçler üzerine etkisini standart kalp cerrahisi ile karşılaştırmayı amaçlamaktadır.

Materyal ve Metod: Asendan aort anevrizması için 28 ° C' da selektif antegrad serebral perfüzyon ve orta derecede hipotermik alt vücut dolaşım durması uygulanan 36 hasta (standart çalışma grubu), konvansiyonel kardiyopulmoner bypass ile standart kalp ameliyatı uygulanan (kontrol grubu) 36 hasta prospektif olarak karşılaştırıldı. Vena kava inferiordan elde edilen kan örneklerinde nitrik oksit, asimetrik dimetilarginin, hidrojen sülfit ve toplam antioksidan kapasite durumu ve laktat seviyeleri incelenmiştir. Klinik sonuçlar ve biyokimyasal parametreler değerlendirildi.

Bulgular: Endotel fonksiyonuyla ilgili biyobelirteçler, asimetrik dimetilarjinin dışındaki gruplar arasında benzer bulundu. Asimetrik dimetilarjinin düzeyleri düşüktü, laktat düzeyleri ise kontrol grubuna göre anlamlı derecede yüksek bulundu. Kontrol gruptaki koroner arter hastalarının baskınlığını nedeniyle çıkan sonuçlarda kafa karıştırıcı bir faktör olarak düşünülerek koroner arter hastaları analiz dışında bırakıldığında, asimetrik dimetilarjinin düzeylerinin, iki alt grup arasında benzer olduğu bulundu.

Sonuç: Çalışma grubundaki düşük plazma asimetrik dimetilarjinin seviyeleri, endotelyal nitrik oksit sentezinde koruyucu bir role sahip olabilir. Koroner arter hastalığı olan hastalar her iki gruptan da dışlandığında, endotel fonksiyonuna bağlı biyobelirteçler her iki grupta da benzerdi. Bu sebeple antegrad serebral perfüzyon ve distal ılımlı hipotermik sirkülatuar arrest tekniğinin kısa dönemde endotel fonksiyonlarını etkilemediğini düşünmekteyiz.

Anahtar Kelimeler: Nitrik oksit, asimetrik dimetil arjinin, hidrojen sülfit, toraks cerrahisi.

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INTRODUCTION

Selective antegrade cerebral perfusion (SACP) with hypothermic lower body circulatory arrest (HLBCA) is defined as a more effective method than deep hypothermic circulatory arrest for cerebral protection during aortic arch aneurysm operations [1]. Until now, promising neurological results have been obtained using this technique. In recent years, to reduce long cardiopulmonary bypass (CPB) times and to avoid adverse effects of profound hypothermia such as coagulation disorders, elevated inflammatory responses, and end-organ dysfunction, SACP with higher core body temperatures (26 to 28°C) have been introduced [2]. Through the proximal right brachial artery with moderate HLBCA at 26 to 28°C, SACP has been used with favorable clinical and neurological outcomes since 1996 in both our clinical setting and in external healthcare centers [2-5]. However, there is a limited number of studies on lower body ischemia or organ functions during moderate HLBCA. Some reports have shown postoperative elevation of serum hepatic, pancreatic enzymes, creatinine, lactate dehydrogenase and C-reactive protein levels without any clinical evidence of organ dysfunction [4-6].

Pathological conditions in vascular bed such as vasospasm, vasoconstriction, excessive thrombosis, and abnormal vascular proliferation result from endothelial dysfunction. Intact endothelial cells play a pivotal role in the regulation of vascular tone and homeostasis by secreting various active substances [7]. Nitric oxide (NO) is one of the main molecules, and decreased synthesis and release of NO results in endothelial dysfunction. It is involved in various physiological and pathological responses, including smooth muscle relaxation, immune regulation, platelet function, and neuronal transmission. Hypoxia stimulates the inducible form of nitric oxide synthase (NOS) which generates higher concentrations of NO and, in hypoxic conditions, reduced production of endothelium-derived NO, and abundant production of inflammatory cells-derived NO occur [8,9]. On the other hand, reoxygenation following hypoxia leads to oxidative stress which also causes endothelial dysfunction by inhibiting endothelium-derived NOS and scavenging NO with superoxide anion [8,9]. Endothelial dysfunction is also linked to elevated levels of asymmetric dimethylarginine (ADMA), an endogenous NOS competitive inhibitor [8,9]. Another gaseous transmitter is hydrogen sulfide (H2S) which interacts with the NO metabolism and oxidative stress in physiological and pathological situations [10]. In addition, increased levels of reactive oxygen species (ROS) lead to endothelial dysfunction via decrease of NO bioactivity. Therefore, total antioxidant levels are critical for a durable endothelial function [11].

It is well-known that CPB and whole-body systemic ischemia/reperfusion (HCA and reperfusion) cause endothelial dysfunction through activation of neutrophils, release of proteolytic enzymes, and products of oxidative stress in vascular structures [12]. However, there is a limited number of data on endothelial function during SACP with moderate LBHCA, despite favorable outcomes reported in the literature. In the present study, we aimed to investigate biomarkers related to endothelial function (NO, H2S, ADMA and total antioxidant capacity (TAC)) during HLBCA with SACP in patients undergoing aortic surgery, compared to standard cardiac surgery under moderate hypothermia and conventional cardiopulmonary bypass (cCPB).

METHODS

In this prospective study, thirty-six consecutive patients operated for an ascending and arcus aortic aneurysm with SACP with moderate HLBCA (study group, SACP+ HLBCA) and another group of 36 patients with either coronary artery disease (CAD) or heart valve disease operated with cCPB (control group, cCPB) between May 2013 and February 2014 were included. The power analysis was carried out to identify the number of patients in both groups. Patients with a recent (<1 week) myocardial infarction, renal, hepatic dysfunction or failure, and/or aortic dissection were excluded. A written informed consent was obtained from each patient. The study was approved by the Ethics Committee (24th May 2013, no: 5) and conducted in accordance with the principles of the Declaration of Helsinki.

Data including patients' characteristics such as age, sex, body mass index, body surface area, presence CAD, diabetes mellitus, and

left ventricular ejection fraction, hematological parameters such as white blood cell and platelet count, biochemical analysis results such as urea, creatinine, aspartate aminotransferase, alanine transaminase, lactate dehydrogenase, gamma-glutamyl transpeptidase, alkaline phosphatase, and total and indirect bilirubin and operative data such as cardiopulmonary bypass (CPB), cross-clamp, and SACP time and body temperature were analyzed. Body temperature was measured with nasopharyngeal probe during the operation. Postoperative intensive care unit (ICU) and hospital stay, duration of ventilator-dependency and complications were also recorded. At the postoperative sixth hour and third day, urea, creatinine, AST, and LDH values were studied.

Surgical Technique

For the patients in the control group, unilateral SACP technique details of the clinic were described in detail previously [4]. For the patients in the control group, standard cannulation (aortic and right atrial cannulas) and CPB procedures were undertaken with similar anesthesia, cardioplegia and monitoring techniques, except for SACP with HLBCA.

Blood Sampling

Blood samples were drawn from the inferior vena cava to obtain venous blood draining from the visceral organs before termination of SACP which corresponds to the removal of cross-clamping in the cCPB group. Plasma lactate levels were evaluated with blood gas analysis at prespecified time points. Blood samples were taken into tubes containing ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich Inc Schnelldorf, Germany) for further analysis (i.e., NO, H2S, ADMA, and TAC levels). All blood samples were centrifuged with 5.000 rpm for 5 min and the plasma obtained were stored at -80°C until analysis which was performed in a single session.

Laboratory Analyses of NO, H2S, ADMA and TAC

The plasma nitrite levels were measured to assess NO production. The measurement was conducted using the spectrophotometric method based on the Griess reaction [13]. This method was modified at Ankara University, Medical Pharmacology Department for 96-well plates. The plasma TAC levels were measured using the method which was described previously [14], based on the reduction of Cu+2 to Cu+1 by the antioxidants in the plasma. Neocuproine (Sigma-Aldrich Inc, Schnelldorf, Germany) was used as a chromogenic agent and a colored complex was formed spectrophotometrically at 455 nm. The plasma H2S levels were measured spectrophotometrically, according to the previously described method based on the measurements of the absorbance of the methylene blue, which produced by the chemical reaction between N, N-dimethyl-p-phenylenediamine and FeCl3, at 670 nm [15]. The ADMA levels were measured using the enzyme-linked immunosorbent assay (ELISA) kit (Immunodiagnostic A.G., Bensheim, Germany) according to the manufacturer's instruction.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows, version 17.0 software (SPSS Inc., Chicago, IL, USA). The power and sample analysis, version 3.0.43 software was used to identify the sample size. Descriptive data were presented in mean ± standard deviation (SD) and median (min-max) values for continuous variables and in number and frequency for categorical variables. The chi-square or Fisher's exact tests were used to compare categorical variables between the groups. The Student's t-test or Mann-Whitney U test were used for continuous variables in independent groups for parametric and non-parametric variables, respectively. A p value of <0.05 was considered statistically significant.

RESULTS

Patient Characteristics and Operative Data

The patients' characteristics and preoperative hematological and biochemical parameters were similar between the groups, except for incidence of CAD in the cCPB group (19.4% vs. 75%, $p<0.001$) (Table 1). Additional procedures in the SACP+ HLBCA group were aortic valve replacement (n = 7), mitral valve replacement (n = 1), aortic valve replacement + coronary artery bypass grafting (n = 2), and coronary artery bypass grafting (n = 2). In the cCPB group, these procedures were isolated coronary artery bypass grafting (n = 27),

mitral valve replacement (n = 5), aortic valve replacement + mitral valve replacement (n = 1), aortic valve replacement (n = 1), septal myectomy + aortic valve repair (n = 1), and modified Bentall procedure (n = 1). For procedure-related data, the CPB time was longer (p = 0.03) and hypothermia was more pronounced in the SACP+ HLBCA group (Table 2).

Table 1. Patients' characteristics, preoperative hematological and biochemical parameters

Variables	Study group (n=36) mean±SD	Control group (n=36) mean±SD	P value
Age (years)	55.03±15.16	56.94±10.16	0.97a
Female Gender n (%)	7 (19.4%)	15 (41.7%)	0.07b
Mass (kg)	76.36±13.28	78.94±15.87	0.52a
Height (cm)	168.44±14.15	166.42±8.68	0.18a
BMI (kg/m2)	26.54±4.38	28.17±4.89	0.21a
BSA (m2)	1.87±0.20	1.85±0.21	0.73*
CAD n (%)	7 (19.4%)	27 (75%)	<0.001b
DM n (%)	2 (5.6%)	6 (16.7%)	0.26c
EF (%)	56.7±9.1	53.2±8.4	0.13a
WBC (K/mm3)	7164.2±1,734.5	7215.8±1,605.6	0.90a
Plt (K/mm3)		250454.5±62,043.9	0.77a
Urea (mg/dl)	37.89±10.84	37.72±8.10	0.90a
Creatinine (mg/dl)	0.95±0.16	0.92±0.18	0.55d
AST (IU/L)	20.08±5.99	19.89±5.84	0.96a
ALT (IU/L)	21.11±11.88	21.92±10.31	0.83a
LDH (IU/L)	380.08±107.13	364.61±71.36	0.43d
GGT (IU/L)	25.47±12.88	24.91±14.88	0.93d
ALP (IU/L)	76.08±18.59	73.06±25.65	0.97a
Total bilirubin (mg/dl)	0.75±0.45	0.63±0.37	0.16d
Direct bilirubin (mg/dl)	0.20±0.94	0.17±0.86	0.27a

BMI: Body mass index, BSA: Body surface area, CAD: Coronary artery disease, DM: Diabetes mellitus, EF: Ejection fraction, WBC: White blood cell, Plt: Platelet, AST: Aspartate amino transferase, ALT: Alanine amino transferase, LDH: Lactate dehydrogenase, ALP : Alkaline phosphatase, GGT: Gamma glutamyl transferase, SD: Standard deviation, kg: kilogram, cm: centimeter, m: meter, mm: millimeter, mg: milligram, dL: deciliter, IU: International unit. a:Student T test; b: Chi-Square test; c:Fischer's exact test; d:Mann Whitney U test, Study group: Selective antegrade cerebral perfusion (SACP)+Hypothermic lower body circulatory arrest (HLBCA), Control group: Conventional cardiopulmonary bypass (cCPB)

Clinical and Biochemical Outcomes

No mortality was observed in either group. However, the patients in the SACP+ HLBCA group required longer mechanical ventilation support postoperatively in the ICU (11.5±4.3 vs. 9.2±3.1 h, p = 0.01), while the length of ICU and hospital stay and complication rates were similar in both groups (p>0.05) (Table 2). Postoperative clinical and biochemical results are given in Table 2.

Table 2. Intraoperative and postoperative variables between study and control groups

Variables	Study group (n=36) mean±SD	Control group (n=36) mean±SD	P value
CPB period (minutes)	113.2±36.71	95.2±33.59	0.03a
Cross clamp (minutes)	74.64±31.86	62.72±26.57	0.09a
ASCP period (minutes)	14.75±3.71	-	
Temperature (°C)	28.00±0.00	31.36±1.64	0.00a
ICU stay (days)	1.3±0.6	1.3±0.5	0.95a
Hospitalization (days)	6.6±1.5	6.7±1.9	0.43a
Mechanical ventilation (hours)	11.5±4.3	9.2±3.1	0.01b
Complications n (%)	6 (30.6%)	5 (13.9%)	0.16c
Intraoperative			
Lactate (mmol/L)	3.90±0.24	2.9±3.4	
Postoperative 6th hour			
WBC (K/mm3)	11656.7±3,552.0	9961.1±2,493.0	0.02b
PLT (K/mm3)	165666.7±39,240.7		0.47b
Urea (mg/dl)	37.9±10.7	34.5±10.0	0.23b
Creatinine (mg/dl)	1.0±0.2	1.0±0.2	0.32b
AST (IU/L)	51.0±25.6	72.7±56.4	0.20b
LDH (IU/L)	692.2±163.4	740.8±300.5	0.92b
Postoperative 3rdday			
WBC (K/mm3)	10459.1±3,957.1	9691.9±2881.0	0.41b
PLT (K/mm3)	162888.9±51006.3		0.12b
Urea (mg/dl)	42.7±15.7	38.7±13.1	0.25b
Creatinine (mg/dl)	0.9±0.3	0.9±0.2	0.32a
Total Bilirubin (mg/dl)	0.8±0.4	0.7±0.5	0.83a
Direct Bilirubin (mg/dl)	0.3±0.2	0.3±0.2	0.97a
AST (IU/L)	45.3±21.9	52.9±29.1	0.25a
ALT (IU/L)	26.3±30.1	35.7±47.1	0.78a
LDH (IU/L)	643.1±188.0	700.3±225.4	0.43a
ALP (IU/L)	66.4±16.7	75,3±32.6	0.21b
GGT (IU/L)	36.4±27.7	45.7±42.7	0.76a

CPB: Cardiopulmonary bypass, ASCP: Antegrade selective cerebral per-

fusion ,ICU: Intensive care unit, WBC: White blood cell, Plt: Platelet, AST: Aspartate amino transferase, ALT: Alanine amino transferase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, SD: Standard deviation, kg: kilogram, cm: centimeter, mm: millimeter, mg: milligram, dL: deciliter, IU: International unit, L: liter, a: Mann Whitney U test ; b:Student T test; c:Fischer's exact test, Study group: Selective antegrade cerebral perfusion (SACP)+Hypothermic lower body circulatory arrest (HLBCA), Control group: Conventional cardiopulmonary bypass (cCPB)

The WBC counts of the SACP+LBHCA group were higher than cCPB group at the postoperative sixth hour (11656.7±3552.0 vs. 9961.1±2493.0 K/mm3, p = 0.02), but returned to normal limits at the postoperative third day (10459.1±3957.1 vs. 9691.9±2881.0 K/mm3, p = 0.41). Other blood test results were similar at the postoperative third day in both groups. Postoperative complications were observed six patients in SACP+ HLBCA group whereas five patients in cCPB group (p = 0.16). Postoperative new onset atrial fibrillation developed in one case in SACP+ HLBCA group and three cases in cCBP group.

Blood Sample Analysis

A statistically significant difference was observed in terms of the ADMA and lactate levels between the groups. Lactate levels were higher (3.90±0.24 vs. 2.9±3.4 mmol /L) and the ADMA levels were lower in the SACP+ HLBCA group (1.30±0.42 vs. 1.62±0.35 µmol/L /L) than cCPB group (<0.001). (Table 2, 3). However, TAC, NO, and H2S levels were similar between the groups (Table 3).

Table 3. Comparison of ADMA, TAC, H2S and NO levels vena cava inferior blood samples and their relevant P values in the study and the control group before and after the patients with coronary artery disease are excluded (Subgroups 1 and 2).

Variables	ADMA (µmol/L)	TAC (µmol/L)	H2S (mM)	NO (µmol/L)
Study group(n=36) mean±SD	1.30± 0.41	814.61± 198.87	30.33± 12.40	48.01± 46.26
Controlgroup (n=36) mean±SD	1.62± 0.36	782.54± 214	28.89± 5.98	71.11± 96.70
P value	<0.001a	0.512b	0.476a	0.604a
Subgroup 1 (n=29) mean±SD	1.30± 0.43	798.26± 202.4	30.10± 13.6	52.55± 49.5
Subgroup 2 (n=9) mean±SD	1.46± 0.33	793.14± 207.1	26.73± 5.6	61.39± 92
P value	0.121a	0.877a	0.355a	0.953a

CAD: Coronary artery disease, ADMA: Asymmetric dimethylarginine, TAC: Total antioxidant capacity; H2S: Hydrogen sulfide, NO: Nitric oxide; SD: Standard Deviation; a:Man Whitney U test; b: Student T test, Study group: Selective antegrade cerebral perfusion (SACP)+Hypothermic lower body circulatory arrest (HLBCA), Control group: Conventional cardiopulmonary bypass (cCPB), Subgroup 1: Study group without coronary artery disease patients, Subgroup 2: Control group without coronary artery disease patients

As high ADMA levels were reported to be associated with atherosclerosis in a previous study [9], a further analysis was performed to exclude patients with CAD from both groups in our study. Study group without CAD patients defined as Subgroup 1 and control group without CAD patients defined as Subgroup 2. The resultant twenty-nine and nine patients in the Subgroup 1 and Subgroup 2, respectively were compared again. The mean values of TAC, NO, H2S, and ADMA levels were found to be similar in both groups (p>0.05).

DISCUSSION

In the present study, in patients with SACP with moderately HLBCA, when patients with coronary artery disease were excluded from both groups we found no statistically significant difference in biomarkers related to endothelial function (NO, H2S, ADMA and TAC), biochemical parameters, and clinical status, compared to the control group.

Deep HCA has been questioned in recent years due to its undesirable systemic effects, such as coagulopathy, increased systemic inflammatory response, higher renal and respiratory failure, neuronal injury, and cerebral microvasculature endothelial dysfunction [12,16]. In a recent meta-analysis, it was found to be associated with higher stroke rates, compared to moderately HCA with SACP [17]. In the past, experimental studies and series were more concerned with time for neuronal injury than time for visceral tissue injury in aortic surgery, as neuronal tissues have a lower threshold for ischemic injury [18]. It is usually not recommended to exceed 30 min of arrest period during HCA without SACP (12 to 15°C). However, with SACP and mild-to-moderate HCA technique, the safe limit of circulatory arrest period for visceral organ protection in the lower body has not been well-established, yet. A mean HLBCA period of 14.8±3.7 min in the study group is a relatively short timeframe to observe any visceral complication at 28°C; however, even with such brief periods, higher lactate levels were observed in the SACP+ HLBCA

group than the cCPB group (3.9±1.5 vs. 2.2±0.8 mmol /L, p< 0.001). This finding suggested that non-oxidative phosphorylation began within this period of moderately HCA in the SACP+ HLBCA group, compared to the control group whose lower bodies were perfused at the meantime. However, we observed no clinical or biochemical alteration in the postoperative period between these groups. In some clinical experimental studies, it was also indicated that the safe limit of circulatory arrest time can be 60 min during moderate hypothermia to avoid visceral ischemia/reperfusion injury and to diminish systemic inflammatory response [16, 19]. Indeed, the present study was planned in such a way that blood samples would have been drawn every 15 min of HLBCA. However, the longest HLBCA period was 28 min in only one patient in the study group. Hence, it still remains unclear whether longer periods of LBHCA may lead to considerable alterations.

Pacini et al. emphasized the importance of temperatures higher than 25°C to be an independent protective factor for isolated liver dysfunction [20]. In accordance with the above mentioned study, no evidence of visceral organ injury was observed in the SACP+ HLBCA group which was performed under moderate hypothermia (28°C). During postoperative follow-up, we observed no clinical impairment of hepatic, renal, or neurological functions, except for longer mechanical ventilation support time in the HLBCA group. This difference can be attributed to the established practices of the ICU specialists about late extubation of aortic arch surgery patients in the ICU. On the other hand, the length of postoperative stay, hospitalization, and complication rates were similar in both groups.

Blood temperature during CPB was shown to be an important indicator of NO production in an experimental study [21]. In the aforementioned study, NO production was higher in a tepid temperature (34°C) than more hypothermic CPB temperatures (28°C). During circulatory arrest and reinstitution of circulation, endothelial hypoxia and subsequent reoxygenation induce oxidative stress with enhanced superoxide generation and diminished NO production, leading to endothelial dysfunction, which is named as ischemia/reperfusion injury [22]. In addition, it is known that, under conditions of hypoxia and acidosis, endothelial NOS activity diminishes, thereby leading to decrease NO levels [23]. These factors may account for the slightly lower NO levels in the study group, although it did not reach statistical significance. The difference might have been more pronounced for longer antegrade cerebral perfusion duration in the present study.

The ADMA levels should be evaluated together with NO levels. The ADMA inhibits the synthesis of NO competitively and plays a crucial role in the initiation of endothelial dysfunction [9]. This close relationship between the ADMA and NO has been studied in a number of studies [24]. In our opinion, this study indicates that low plasma levels of endogenous NO synthase inhibitor ADMA in the aneurysms group during short periods of moderately HLBCA may have a protective role in endothelial NO synthesis. We also know that chronic serum ADMA level increase is to related CAD. In the present study, the ADMA levels were lower in the study group and higher in the control group. As the number of patients with cCAD was higher in the control group, we decided to exclude patients with CAD from both groups to rule out the predominance of CAD patients in one group as a confounding factor. When patients with cCAD were eliminated, we observed no difference regarding ADMA levels between the two subgroups.

Hydrogen sulfide has been identified on vascular endothelium as the third endogenous signaling gasotransmitter (after NO and CO) which has many biological functions such as metabolic modulation, vasodilatation, and angiogenesis [10,25]. Moreover, H2S exerts powerful antioxidative, anti-inflammatory, cytoprotective, and organ-protective effects, particularly in kidneys [26-28]. While physiological concentrations of H2S have cytoprotective effects, its high concentrations are associated with cytotoxic effects, as H2S itself is already toxic [10]. In addition, H2S plays a critical role for the persistence of the endothelial system function [29]. Many experimental studies have shown protective effects of H2S on liver, kidney, lung, and heart after ischemia/reperfusion injury [26,30]. Plasma concentration of H2S, therefore, may be a good indicator for endothelial function after ischemia/reperfusion injury. Furthermore, H2S also works as an antioxidant owing to its thiol

group that allows reduction of disulfide bonds and radical scavenging [10]. In the present study, we found no significant difference between the groups in terms of the H2S levels. This result may indicate that short periods of HLBCA (14 min), as in our study, may affect endothelial function not more than cCPB.

The amount of ROS is expected to increase during moderately hypothermic HLBCA as a result of reperfusion injury. The level of antioxidant components of plasma is of utmost importance for the continuity of endothelial function, since ROS can damage cells and lead to endothelial dysfunction [11]. In the present study, there was no statistically significant difference in the TAC levels between the two groups. On the other hand, higher lactate levels in the study group suggested that anaerobic glycolysis began early during HLBCA due to insufficient oxygen supply. In addition, similar serum TAC levels indicated that HLBCA had no additional harmful effect on the antioxidative status during this short period of ischemia, compared to cCPB.

Limitations

Nonetheless, the present study has some limitations. First, ischemic time (SACP+ HLBCA period) of the study group was most probably short to observe any endothelial dysfunction. Long duration of SACP with moderately HLBCA was not desired by surgeons to avoid clinical complications in aortic surgery. Although the study was originally designed to compare longer HLBCA times as well, we observed no complex arch pathologies that would require longer circulatory arrest periods for a durable and complete repair. Therefore, the longest SACP time was 28 min in only one patient in the study group. Second, it would have been more appropriate to compare this technique with other aortic surgery techniques, such as deep HCA. However, we have mostly abandoned the use of such techniques in our clinic and use it on rare occasions, such as pediatric patients who are considered ineligible candidates. Third, the number of patients with cCAD in the control group exceeded that of in the study group. When these patients were excluded for subgroup analysis, the resultant patient numbers in both subgroups were not similar, indicating in a weak statistical comparison. Fourth, Blood samples for analysis (i.e., NO, H2S, ADMA, and TAC levels) were drawn from the inferior vena cava to obtain venous blood draining from the visceral organs before termination of SACP which corresponds to the removal of crossclamping in the cCPB group. However, if the sampling had been drawn after initial reperfusion, blood level tests would have been more meaningful for endothelial function. Finally, cCPB temperature and cCPB periods were longer in the study group due to the more complex nature of the aortic surgery technique, compared to other operations in the control group.

CONCLUSION

In conclusion, despite these limitations, we consider that endothelial functions are not affected adversely during short periods of moderately HLBCA. Low plasma levels of ADMA, endogenous NO synthase inhibitor, in the study group may have a protective role in endothelial NO synthesis. When patients with CAD were excluded from both groups to rule out the predominance of CAD patients in the control group as a confounding factor, biomarkers related to endothelial function were found to be similar in both subgroups during short periods of HLBCA. This favorable result is also true for clinical results, compared to other cardiac operations with cCPB. However, further large-scale studies are needed to confirm our findings and to investigate visceral organ status in longer periods of SACP with moderately HLBCA.

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REFERENCES

1. Kazui T. Update in surgical management of aneurysms of the thoracic aorta. Rinsho Kyobu Geka 1986;6:7-15. PMID: 9423028
2. Zierer A, El-Sayed Ahmad A, Papadopoulos N, Moritz A, Diegeler A, Urbanski PP. Selective antegrade cerebral perfusion and mild (28°C-30°C) systemic hypothermic circulatory arrest for aortic arch replacement: results from 1002 patients. J Thorac Cardiovasc Surg. 2012;144:1042-49. PMID: 22967770
3. Ozatik MA, Kucuker SA, Tülüce H, Sartiaş A, Sener E, Karakaş S, et al. Neurocognitive functions after aortic arch repair with right brachial artery perfusion. Ann Thorac Surg. 2004;78:591-95. PMID: 15276528
4. Ozen A, Altıntaş G, Unal EU, Yilmaz M, Ozen S. Factors affecting long-term survival of patients undergoing aortic surgery using the antegrade selective cerebral perfusion technique. Turk Gogus Kalp Dama. 2014;22:13-18. doi:10.5606/tgkdc.dergisi.2014.8205.
5. Leshnower BG, Thourani VH, Halkos ME, Sarin EL, Keeling WB, Lamias MJ, et al. Moderate versus deep hypothermia with unilateral selective antegrade cerebral perfusion for acute type A dissection. Ann Thorac Surg. 2015;100:1563-68. PMID: 26233273
6. Khaladj N, Shrestha M, Meck S, Peterss S, Kamiya H, Kallenbach K, et al. Hypo-

thermic circulatory arrest with selective antegrade cerebral perfusion in ascending aortic and aortic arch surgery: a risk factor analysis for adverse outcome in 501 patients. J Thorac Cardiovasc Surg. 2008;135:908-14. PMID: 18374779

7. Landmesser U, Hornig B, Drexler H. Endothelial function: A critical determinant in atherosclerosis. Circulation. 2004;109:27–33. PMID: 15173060
8. Ho JJ, Man HS, Marsden PA. Nitric oxide signaling in hypoxia. J Mol Med. 2012;90:217-31. Doi: 10.1007/s00109-012-0880-5.
9. Özden Işıklar Ö, Mutaf I. Asymmetric Dimethylarginine and it's Clinical Significance. Türk Klinik Biyokimya Derg. 2010;8:75-89
10. Wagner F, Asfar P, Calzia E, Radermacher P, Szabó C. Bench-to-bedside review: Hydrogen sulfide the third gaseous transmitter: applications for critical care. Crit Care. 2009;13:213-22. PMID: 19519960
11. Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, et al. Age-related reduction of NO availability and oxidative stress in humans. Hypertension. 2001;38:274-79. PMID: 11509489
12. Cooper WA, Duarte IG, Thourani VH, Nakamura M, Wang NP, Brown WM, et al. Hypothermic circulatory arrest causes multisystem vascular endothelial dysfunction and apoptosis. Ann Thorac Surg. 2000;69:696–703. PMID: 10750746
13. Navarro-Gonzalvez J, Garcia-Benayas C, Arenas J. Semiautomated measurement of nitrate in biological fluids. Clin Chem. 1998;44:679-81. PMID: 9510886
14. Usanmaz SE, Demirel Yilmaz E. A microplate based spectrophotometric method for the determination of the total antioxidant capacity of human plasma: modified cupric reducing ability assay. Fund & Clin Pharmacol. 2008;22:67.
15. Zhang H, Moochhala SM, Bhatia M. Endogenous hydrogen sulfide regulates inflammatory response by activating the ERK pathway in polymicrobial sepsis. J Immunol. 2008;181:4320-31. PMID: 18768890
16. Kamiya H, Hagl C, Kropivnitskaya I, Böthig D, Kallenbach K, Khaladj N, et al. The safety of moderate hypothermic lower body circulatory arrest with selective cerebral perfusion: a propensity score analysis. J Thorac Cardiovasc Surg. 2007;133:501-09. PMID: 17258589
17. Tian DH, Wan B, Bannon PG, Misfeld M, LeMaire SA, Kazui T, et al. A meta-analysis of deep hypothermic circulatory arrest versus moderate hypothermic circulatory arrest with selective antegrade cerebral perfusion. Ann Cardiothorac Surg. 2013;2:148-58. PMID: 23977575
18. Luehr M, Bachet J, Mohr FW, Etz CD. Modern temperature management in aortic arch surgery: the dilemma of moderate hypothermia. Eur J Cardiothorac Surg. 2014;45:27-39. PMID: 23628950
19. Pacini D, Leone A, Di Marco L, Marsilli D, Sobaih F, Turci S, et al. Antegrade selective cerebral perfusion in thoracic aorta surgery: safety of moderate hypothermia. Eur J Cardiothorac Surg. 2007;31:618-22. PMID: 17254793
20. Pacini D, Pantaleo A, Di Marco L, Leone A, Barberio G, Murana G, et al. Visceral organ protection in aortic arch surgery: safety of moderate hypothermia. Eur J Cardiothorac Surg. 2014;46:438-43. PMID: 24493060
21. Ohata T, Sawa Y, Kadoba K, Kagisaki K, Suzuki K, Matsuda H. Role of nitric oxide in a temperature dependent regulation of systemic vascular resistance in cardiopulmonary bypass. Eur J Cardiothorac Surg. 2000;18:342-47. PMID: 10973545
22. Özcan G, Şahin EA, Demirel Yilmaz E. Effect of Nitric Oxide on Cardiac Functions: Review. Turkiye Klinikleri J Cardiovasc Sci. 2016;28:99-117. doi: 10.5336/cardiosci.2016-53254
23. Omar SA, Webb AJ. Nitrite reduction and cardiovascular protection. J Mol Cell Cardiol. 2014;73:57-69. PMID:24486197
24. Xuan C, Tian QW, Li H, Zhang BB, He GW, Lun LM. Levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, and risk of coronary artery disease: A meta-analysis based on 4713 participants. Eur J Prev Cardiol. 2016;23:502-10. PMID: 25956428
25. Szabo C. Medicinal chemistry and therapeutic applications of the gasotransmitters NO, CO, and H2S and their prodrugs. In: Burger's Medicinal Chemistry and Drug Discovery. Hoboken, NJ: John Wiley & Sons, 2010:265–368. doi:10.1002/0471266949.bmc178.
26. Dursun M, Otunctemur A, Ozbek E, Sahin S, Besiroglu H, Ozsoy OD,et al. Protective effect of hydrogen sulfide on renal injury in the experimental unilateral ureteral obstruction. Int Braz J Urol. 2015;41:1185-93. PMID: 26742979
27. Bełtowski J, Hypoxia in the renal medulla: implications for hydrogen sulfide signaling. J Pharmacol Exp Ther. 2010;334:358-64. PMID: 20427475
28. Calvert JW, Coetzee WA, Lefer DJ. Novel insights into hydrogen sulfide mediated cytoprotection, Antioxid. Redox Signal. 2010;12:1203-07. PMID: 19769484
29. Altaany Z, Moccia F, Munaron L, Mancardi D, Wang R. Hydrogen sulfide and endothelial dysfunction: relationship with nitric oxide. Curr Med Chem. 2014;21:3646-61. PMID: 25005182
30. Sivarajah A, Collino M, Yasin M, Benetti E, Gallicchio M, Mazzon E, et al. Antiapoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial I/R. Shock. 2009;31:267-74. PMID: 18636044