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European Journal of Cardio-thoracic Surgery 17 (2000) 723–728

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

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Expression of adhesion molecules and cytokines after coronary artery bypass grafting during normothermic and hypothermic cardiac arrest

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Received 8 December 1999; received in revised form 8 February 2000; accepted 15 February 2000

Abstract

Objective: Cardiac surgery with cardiopulmonary bypass (CPB) results in vascular injury and tissue damage which involves leukocyte-endothelial interactions mediated by cytokines and adhesion molecules. This study was designed to demonstrate the effect of normothermic and hypothermic CPB to cytokine and soluble adhesion molecule levels in adults and to determine whether these levels correlate to the patients postoperative course. **Design and patients**: In 25 patients after normothermic and in 25 patients after hypothermic coronary artery bypass grafting with cardiopulmonary bypass (CPB), blood samples for cytokine and soluble adhesion molecule analysis were taken preoperatively, 24, 36, 48 h, and 6 days postoperatively. Soluble adhesion molecules (sE-selectin, sICAM-1) were measured by ELISA and cytokines (TNF-α, IL-6, IL-8) by chemilumenscent-immunoassay. Clinical data were collected prospectively. **Results**: Postoperatively, adhesion molecule and cytokine levels were significantly elevated after CPB. Mean plasma levels of sICAM-1 was 2.4-fold higher after 6 days. Mean plasma concentration of sE-selectin peaked after 48 h with a 2-fold increase compared to normothermic conditions. In the hypothermia group sICAM-1, sE-selectin, IL-6, and IL-8 showed significantly higher levels ($P < 0.0057$, $P < 0.0012$, $P < 0.0419$, $P < 0.0145$) after 24 h compared to the normothermia group. No clinical differences were seen. **Conclusion**: Adhesion molecules and cytokines are elevated after CPB. Patients after hypothermic CPB show significant higher sICAM-1, sE-selectin, IL-6, and IL-8 levels after 24 h compared to normothermic conditions. These results are mainly due to longer CPB and crossclamp times but do not alter the patient's postoperative course.

Keywords: Cardiac surgery; Ischemia/reperfusion; Adhesion molecules; Cytokines

1. Introduction

Adhesion of leukocytes to endothelium and migration into tissues are important events in the inflammatory response to cardiopulmonary bypass. These leukocyte-endothelial interactions are mediated by various adhesion molecules on the surface of activated leukocytes, platelets and endothelium. In addition to membrane-bound molecules, soluble isoforms are found in the circulating blood. Cardiac operations using cardiopulmonary bypass (CPB) are associated with release of various cytokines, activation of the coagulation cascade, and increased plasma levels of circulating adhesion molecules [1]. An increase in soluble adhesion molecules results either from increased expression on activated endothelial cells or from increased proteolytic cleavage of endothelial-bound forms secondary to endothelial cell damage [2]. Thus, high levels of soluble adhesion molecules may serve as markers for activated or damaged endothelium. The role of endothelial selectin (E-selectin) and intercellular adhesion molecule-1 (ICAM-1) consist of initiating rolling and mediating firm adherence of leukocytes to the endothelium with subsequent migration into tissues [2].

A strategy that would inhibit the migration of leukocytes across the endothelial layer would blunt the inflammatory response induced by CPB and prevent the tissue ischemia-reperfusion injuries. Different therapeutic methods have been proposed by us and others including salicylates, complement receptor or adhesion molecule blocking agents [3,4]. Other concepts to attenuate myocardial reperfusion injury consist of inhibiting cytokine release and/or induction

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1010-7940/00/$ - see front matter
PII: S1010-7940(00)00401-2

of adhesion molecule expression by establishing hypothermic conditions during CPB or removing circulating cytokines by instituting modified ultrafiltration [5].

This study was designed to demonstrate the effect of normothermic and hypothermic CPB to soluble adhesion molecule levels in adults and to determine whether these levels correlate to the patients postoperative course.

2. Methods

2.1. Patient groups

Patients recruited for this prospectively controlled, randomized study were undergoing elective coronary artery bypass grafting using normothermic or hypothermic cardiopulmonary bypass. Between July 1997 and December 1998 we enrolled 25 patients (mean age 58.5 years) in the normothermia and 25 patients (mean age 55.9 years) in the hypothermia group. Informed consent was obtained from each patient according to the protocol of the ethics committee of the hospital.

2.2. Operative techniques

Cardiopulmonary bypass was performed with a Stöckert roller pump system (Stöckert Instrumente GmbH, Munich, Germany) and a Shiley-Dideco Maxima hollow fiber oxygenator (Dideco, Mirandola, Italy). Myocardial protection was accomplished using cold cristalloid cardioplegia with an initial dose of 30 ml/kg repeated every 20 min. Before aortic declamping, 15 ml/kg warm blood cardioplegia ('hot shot') was administered. The operations were performed under normothermic (34°C) by only one surgeon and under hypothermic conditions (24–26°C) by three different surgeons according to the usual practice of our clinic. Rewarming was achieved using the heat-exchange oxygenator, warming blanket, and heated humidified gases to reach a rectal temperature of >34°C before terminating CPB. The use of intra- and postoperatively medications did not differ between the two groups.

2.3. Blood sampling and analysis

Blood samples (10 ml) for measuring cytokines, soluble E-selectin (sE-selectin), soluble ICAM-1 (sICAM-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor-α (TNF-α) were obtained from a venous line of the patient at the following times: at induction of anesthesia, 24, 36 (only sE-selectin and sICAM-1), 48 h and 6 days after CPB. Blood samples were allowed to coagulate, centrifuged for 20 min at 4°C and the serum was aliquoted and stored at −70°C [2]. Soluble adhesion molecules in the serum were measured using commercially available ELISA kits (R&D Systems, Abingdon, UK). Standards of known concentration were run in parallel together with a control serum. The optical density (OD) was read at 450 nm with a correction wavelength set to 630 nm. The absorbance was plotted against a standard curve of known concentrations and expressed as ng/ml. The values were corrected by hemodilution using hematocrit. Cytokines (TNF-α, IL-6, IL-8) were analyzed by the technique of a solid-phase, two-site chemilumenscent enzyme immunometric assay (Immulite, EURO/DPC Ltd, Gwynedd, UK).

2.4. Clinical variables

Patient demographic data and medical history were collected prospectively, as well as the postoperative course including intubation time, blood loss, ICU stay, occurrence of atrial fibrillation or pulmonary infection requiring antibiotic treatment and length of hospital stay.

2.5. Statistics

Data were processed with Statview software (Abacus Concepts Inc., Berkeley, CA). All data were expressed as mean with one standard deviation and were graphically presented as bar charts. Intergroup comparison was performed with the Mann–Whitney test for unpaired data. Intragroup comparison was done using the Wilcoxon test for paired data. Normally distributed values between groups were compared by the student's *t*-test or in case of repeated measurements over the time by analysis of variance (ANOVA) for repeated measures. Non-normally distributed data were compared either with the Fisher's exact test or the Mann–Whitney *U*-test with Bonferroni correction.

3. Results

In all patients sICAM-1 and sE-selectin were significantly elevated after CPB. The mean plasma level of sICAM-1 increased up to 2.4-fold (635 ng/ml) after 6 days compared to the preoperative sample (262 ng/ml). Mean plasma concentrations for sE-selectin showed a peak after 48 h of 75 ng/ml with a slight regression after 6 days representing an increase of at least 2-fold compared to the control sample (45 ng/ml). The time course of plasma level of soluble adhesion molecules differed between normothermic and hypothermic CPB demonstrating significantly higher sICAM-1 ($P = 0.0057$, $P = 0.0049$) and sE-selectin ($P = 0.0012$, $P = 0.015$) levels in hypothermia compared to normothermic conditions after 24 and 36 h. However, there were no differences seen in sICAM-1 and sE-selectin expression 6 days after the operation between normothermia and hypothermia although significantly higher than the preoperative values (Fig. 1). In line with the adhesion molecules cytokine levels (IL-6, $P = 0.0419$, IL-8 $P = 0.0145$) were significantly higher in the hypothermia group at 24 h postoperatively (Fig. 2).

Regarding the demographic data the two groups were not significantly different for age and gender although men and women were unevenly distributed. There were more men

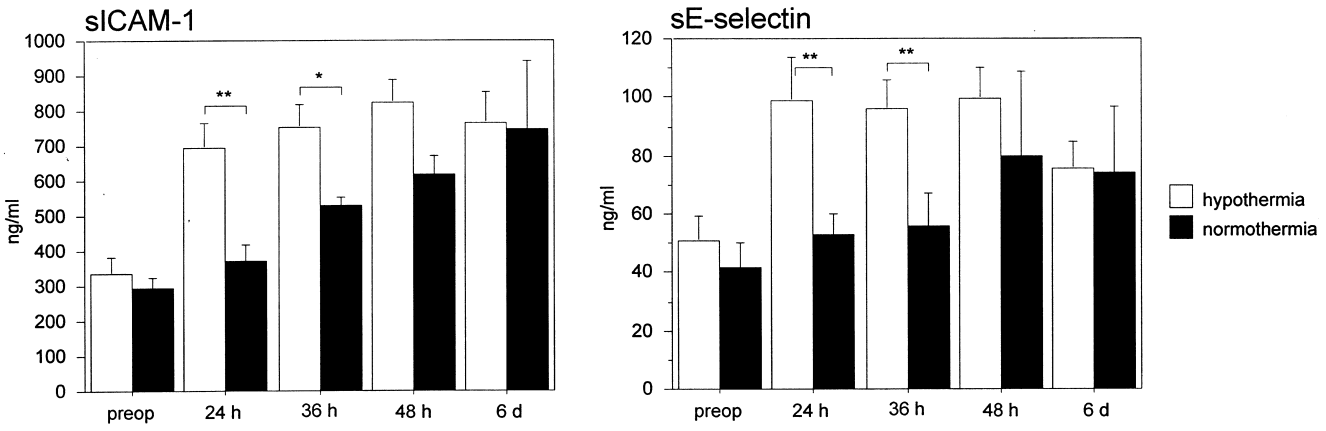

Fig. 1. Expression of sICAM-1 and sE-selectin after normothermic and hypothermic cardiopulmonary bypass (CPB) at different time intervals (*$P < 0.05$, **$P < 0.01$), values are mean ± standard deviation.

undergoing coronary artery bypass than women (38 men vs. 12 women). However, the only difference in the operative variables between the two groups was the mean total cardiopulmonary bypass and the mean crossclamp time which were significantly shorter in the normothermia group (74 min/37 min) compared to the hypothermia group (101 min/62 min). This discrepancy was mainly due to the surgeon's experience in the hypothermia group.

The postoperative course in both groups showed no significant difference in intubation time, infection rate, length of ICU or hospital stay (Table 1). Postoperative atrial fibrillation was more frequent in normothermia compared to hypothermia (9 vs. 3 patients, NS). Blood loss was similar in both groups. Age, gender and pump or crossclamp time showed no correlation to adhesion molecule or cytokine levels.

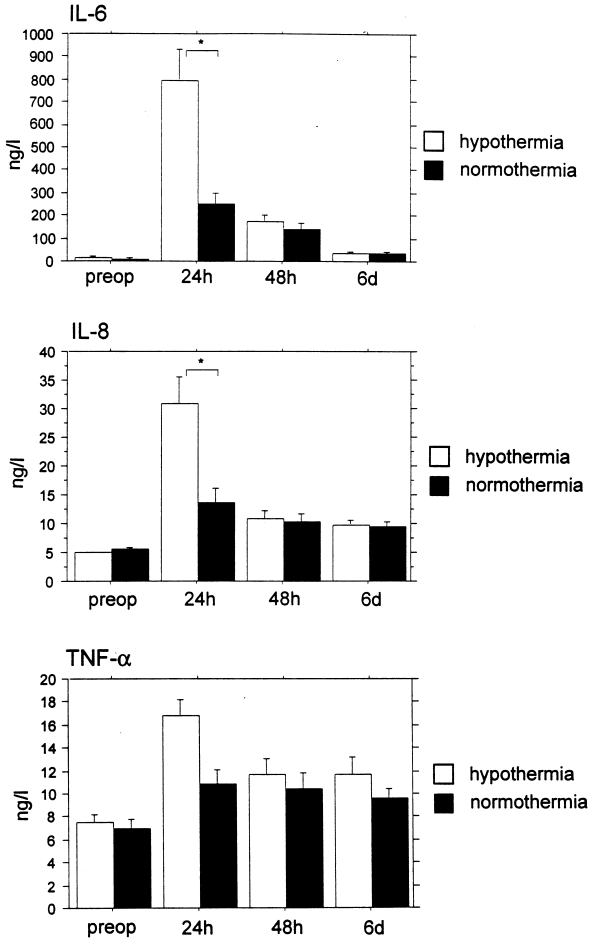

Fig. 2. Expression of cytokines after normothermic and hypothermic cardiopulmonary bypass (CPB) at different time intervals (*$P < 0.05$), values are mean ± standard deviation.

4. Discussion

Plasma levels of soluble adhesion molecules sICAM-1 and sE-selectin, as well as IL-6 and IL-8 are significantly higher in hypothermic cardiopulmonary bypass compared to normothermic conditions. An increase in soluble adhesion molecules results either from increased expression in activated endothelial cells or from increased proteolytic cleavage of endothelial-bound forms secondary to endothelial cell injury [6]. Increased serum levels of sE-selectin and sICAM-1 not only may serve as markers for the degree of vascular cell injury, but also retain its ability to bind to CD11b/CD18 and may compete with the membrane-bound ICAM-1 for leukocyte adhesion, thus preventing neutrophil-induced tissue damage [7]. The increased expression of myocardial ICAM-1 and E-selectin has been demonstrated in several studies [8], as well as the role of early ICAM-1 induction in neutrophil-mediated myocardial damage after cardiopulmonary bypass [9,10]. In vitro studies revealed induction of E-selectin and ICAM-1 mRNA in human umbilical vein endothelial cells by IL-1 and TNF-α within 1–4 h [11]. In addition isolated canine myocardium have been shown to express ICAM-1 on stimulation either with cytokines or with postreperfusion cardiac

Table 1
Clinical variables in normothermic and hypothermic bypass patients[a]

	Normothermic CPB	Hypothermic CPB	*P*-value
Age (years)	60.8 ± 8.9	56.7 ± 12.4	NS
Gender (female/male)	4/21	8/17	NS
Bypass time (min)	74 ± 22	101 ± 33	0.0012
Crossclamp time (min)	37 ± 8	63 ± 27	0.0002
Intubation (days)	1	2.1 ± 4.8	NS
ICU (days)	2.2 ± 3.4	3.6 ± 5.8	NS
Pulmonary infection (patients)	4	9	NS
Atrial fibrillation (patients)	9	3	NS
Blood loss (units)	1.3 ± 0.3	1.8 ± 0.7	NS
Hospital stay (days)	10.9 ± 4.4	13.7 ± 8.2	NS

[a] CPB, cardiopulmonary bypass; NS, not significant; values are mean ± standard deviation.

lymph. All these observations suggest that ICAM-1 induction might occur in atrial myocytes, as well as in vascular endothelium following ischemia and reperfusion [8].

Cardiopulmonary bypass is associated with increased plasma levels of circulating adhesion [12]. However, no upregulation of those molecules could be found in major non-cardiac operation compared to patients undergoing elective coronary artery bypass grafting [13]. E-selectin is particularly interesting because it is found only on activated endothelium, in contrast to other adhesion molecules which have a wider tissue distribution. The detection of soluble E-selectin in the blood has therefore to be taken as conclusive evidence of endothelial cell activation.

In the present study, the time course observed for adhesion molecule expression after CPB showed a characteristic pattern with a rapid and sustained increase of sE-selectin and sICAM-1 levels after surgery. As expected from prior own in vitro studies E-selectin had a slightly earlier peak level at 48 h in normothermia followed by an immediate decline but without normalization to baseline levels after 6 postoperative days [14]. In contrast, ICAM-1 increased constantly showing highest levels 6 days postoperatively. Under hypothermic conditions however, changes in ICAM-1 and E-selectin levels were markedly different from those under normothermic conditions. Both molecules showed significantly higher levels at 24 and 36 h after CPB compared to normothermic conditions approximating after 6 days. The persistent high plasma levels of adhesion molecules for at least 6 postoperative days in adult patients after cardiopulmonary bypass are striking and may generally be explained by continuous induction of cytokines throughout the whole hospital stay until discharge.

Three different factors may possibly be responsible for higher adhesion molecule and cytokine production after hypothermic cardiopulmonary bypass: cardiopulmonary bypass time, ischemia time and bypass temperature. According to the known facts of cytokine biology, the immediate early effects of CPB is the activation of complement factors, such as C3a and C5a, which alone or with other factors result in induction of adhesion molecules. We believe that a continuous stimulus does lead to higher cytokine levels, and therefore do consider longer cardiopulmonary bypass time the most important factor for those significantly higher adhesion molecule and cytokine levels. The same mechanism probably may not be applied for longer ischemia time. Based on our previous laboratory results with human endothelial cell we found that ischemia alone simulated in a hypoxic chamber does not lead to significantly higher induction of adhesion molecules compared to normoxia. However, adding TNF-α to the medium mimicking bypass conditions does serve as a potent trigger with consecutive major adhesion molecule upregulation [15]. As we showed in a previous study the complement component C5a and TNF-α have a synergistic effect on the induction of endothelial E-selectin and ICAM-1 expression which clearly is dose-dependent [16]. These results indicate that ischemia itself without the presence of cytokines may only to a certain extent be responsible for excessive adhesion molecule expression. Certainly, those two factors are time-dependent and therefore directly correlated to the surgeon's technical experience.

The temperature during cardiopulmonary bypass procedures is still a subject of ongoing debate [17] although moderate hypothermic or even normothermic cardiopulmonary bypass in cardiac surgery has gained more and more acceptance in recent years. However, the extent of adhesion molecule or cytokine upregulation under normothermic and hypothermic conditions has always been controversial [18]. Boldt and coworkers reported of a study in which no significant differences in adhesion molecule expression except for P-selectin was found between normothermia and hypothermia in patients undergoing coronary artery bypass grafting in a matched patient population [19]. The reasons for the higher sP-selectin levels under hypothermic conditions could only be speculated on that an increase either results from the inflammatory process itself or from changes of intravascular coagulation, i.e. alterations in platelet function and thrombin generation [19]. Interestingly, it has also been reported that secretion of high levels of sP-selectin appear to prevent the adhesion process of neutrophils via CD11b to endothelium by blocking the TNF-mediated activation of the β_2-integrin complex [10]. From a cardiac surgical standpoint hypothermia to reduce myocardial oxygen demand has been standard practice for years. Lichtenstein et al. reassessed the need for hypothermia in cardiac surgery and highlighted its adverse effects [20]. It has been demonstrated that the decrease in oxygen demand from the working to the arrested heart is mainly due to elimination of electromechanical activity, rather than cooling. Hypothermic arrested hearts require only about 5% less oxygen than arrested and normothermic

hearts. In contrast, the difference of oxygen consumption between an arrested and working state exceeds 95% so that the need for hypothermia becomes questionable [20]. It is known that hypothermia changes the activity of important enzymes, membrane stability and adenosine triphosphate generation and utilization which might lead to disturbed cellular function in in vivo conditions [21]. Even changes in ultrastructure and cellular edema were reported leading to cellular necrosis after hypothermia and rewarming [22,23]. Supported by a study of Frering et al. in which no systemic release of TNF-α and IL-1 was found in normothermic CPB [24], we believe that those changes seen in hypothermic CPB may be due to cell damage by the cooling and rewarming process and is reflected with excessive cytokine production and subsequent adhesion molecule expression. Unfortunately, we do not have any data to confirm this hypothesis.

The pathophysiology of these responses to cardiopulmonary bypass are gradually becoming clarified. Approaches can be described to prevent or at least modify this inflammatory cascade based on the understanding of the whole mechanism. Administration of scavenging agents could be used to reduce the activation and release of the free radical response to CPB [25]. Other studies have been undertaken to demonstrate the efficacy of a modified ultrafiltration in removing inflammatory mediators generated during CPB [5]. Even more promising might be new drugs to prevent complement activation and cytokine generation to combat inflammation and bleeding complications. The benefits of an eventual use of anti-adhesion molecule or anti-complement receptor therapy in a clinical setting may, however, be potentially dangerous, because altering the immunological response by modifying the function of adhesion molecule or the complement cascade may attenuate the injury caused by host defense cells. Our results of still elevated adhesion molecule levels after 6 days imply that any therapeutic treatment should be performed for at least this time period until patient discharge.

This study was conducted in a randomized patient population undergoing elective coronary artery bypass grafting without emergency operations and several study limitation have to be noted. First, assessing the diagnostic or physiological significance of in vivo plasma levels may be difficult due to discrepancy between average levels in the blood and more accurate levels in the reperfused organ, i.e. myocardium, brain [2]. High levels may also reflect the rate of cellular adhesion molecule synthesis and subsequent surface expression, the rate of cleavage from the cell surface, or simply decreased clearance. Second, since we only recorded postoperative clinical parameters for maximal 12 days we cannot rule out that those higher inflammatory mediators do not have any long term detrimental effects, i.e. for the endothelium in vein grafts.

The higher plasma levels of adhesion molecules and cytokines in hypothermia is only explained by longer pump or crossclamp times and possibly due to the cooling and rewarming process itself. Since no significant clinical changes were seen between normothermic and hypothermic CPB we believe that intermittently higher adhesion molecule and cytokine levels do not alter the patients immediate postoperative course.

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