The effects of cardiopulmonary bypass temperature on inflammatory response following cardiopulmonary bypass


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Abstract

Objectives: The inflammatory response to cardiopulmonary bypass is believed to play an important role in end organ dysfunction after open heart surgery and may be more profound after normothermic systemic perfusion. The aim of the present study was to investigate the effects of cardiopulmonary bypass temperature on the production of markers of inflammatory activity after coronary artery surgery.

Methods: Forty-five low risk patients undergoing elective coronary artery surgery were prospectively randomized into three groups: hypothermia (28°C, n = 15), moderate hypothermia (32°C, n = 15), and normothermia (37°C, n = 15). All patients received cold antegrade crystalloid cardioplegia and topical myocardial cooling with saline at 4°C. Serum samples were collected for the estimation of neutrophil elastase, interleukin 8, C3d, and IgG under ice preoperatively, 5 min after heparinisation, 30 min following start of CPB, at the end of CPB, 5 min after protamine administration, and 4, 12 and 24 h postoperatively.

Results: Patients were similar with regard to preoperative and intraoperative characteristics (age, sex, severity of symptoms, number of grafts performed, aortic cross clamp time, cardiopulmonary bypass time). Neutrophil elastase concentration increased markedly as early as 30 min after the onset of cardiopulmonary bypass and peaked 5 min after protamine administration. Levels were not significantly different between the three groups. A similar finding was apparent for C3d release. Interleukin 8 concentrations also demonstrated a considerable increase related to cardiopulmonary bypass in all groups, but there was a significantly more rapid decline in interleukin 8 concentrations in the normothermic group in the postoperative period. Eluted IgG fraction showed a much earlier peak concentration than the other markers, occurring within 30 min of the start of cardiopulmonary bypass. Levels reached a plateau, before declining soon after the end of bypass and remained higher than preoperative values at 24 h. There was no difference between the three groups. The cumulative release of all markers was calculated from the concentration-time curves, and was not statistically different between groups. Conclusion: Normothermic systemic perfusion was not shown to produce a more profound inflammatory response compared to hypothermic and moderately hypothermic cardiopulmonary bypass. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cardiopulmonary bypass; Coronary artery surgery; Inflammatory mediators

1. Introduction

The deleterious effects of cardiopulmonary bypass are believed to be related to activation of neutrophils, complement, interleukin production, free radical generation and a wide variety of other responses collectively known as the systemic inflammatory response. In the present investigation, a variety of surrogate markers of the inflammatory response were measured to determine the role of perfusion temperature on the magnitude and temporal nature of this response. Neutrophil elastase is an endopeptidase that has been used as a marker of neutrophil activation by a number of workers [1,2] and may be responsible for neutrophil induced endothelial injury [3], particularly in the lung. Activation of the common complement pathway leads to the conversion of C3 to C3a and a fragment known as C3d. This fragment was measured to assess the degree of complement activity. Interleukin 8 (IL-8) is a potent neutrophil chemotactic and activating factor that may play a central role in the pathogenesis of adult respiratory distress syndrome [4] and reperfusion injury to the myocardium [5]. IgG can be denatured by neutrophil derived reactive oxygen species and eluted IgG fraction can be quantified to estimate the presence of free radical activity [6,7].
2. Material and methods

Forty-five patients (mean age 64 ± 8 years, 35 males) undergoing primary isolated coronary revascularisation on an elective basis were randomized into three groups depending upon the systemic perfusion temperature employed (28, 32 and 37°C). Patients with diabetes mellitus, or unstable angina were excluded from the investigation.

Anaesthetic techniques were standardised for all patients. Thiopentone (1–3 mg/kg) was used for induction, 3–5 μg/kg Fentanyl and volatile agents delivered in 50% air–O_2 mixture for maintenance, and midazolam infusion given during CPB. Neuromuscular blockade was achieved by 0.1–0.15 mg/kg Pancuronium bromide, and ventilation was adjusted to maintain normocapnoea. Alpha stat acid-base management was adopted.

Initial heparinisation was accomplished with 3 mg/kg body weight of heparin and was supplemented as needed to maintain an activated clotting time of 480 s. Preparation for CPB consisted of ascending aortic cannulation and two stage venous cannulation via the right atrial appendage. A standard CPB circuit was used in all patients including PVC tubing (Sorin Biomedica UK Ltd, Midhurst, UK), a Cobe roller pump (Cobe, Lakewood, CO), hollow fibre membrane oxygenator (Monolyth, Sorin Biomedica Cardio, Saluggia, Italy), and a 40μ arterial line filter (Sorin Linea ABF 40). The extra corporeal circuit was primed with 1000 ml Hartmann’s solution, 500 ml Gelofusine (B Braun Medical Ltd., Emmenbrücke, Switzerland) and 60 mg of heparin. Hypothermic and moderate hypothermic CPB were conducted with the perfusate at the appropriate temperature to reach a nasopharyngeal temperature of 28 or 32°C respectively. Patients in the normothermic group were actively warmed to maintain a nasopharyngeal temperature of 37°C throughout the period of CPB. Non-pulsatile perfusion was used throughout the procedure, and flow was maintained at 2.4 l/m²/min for the normothermic group, and was reduced to 2.0 l/m²/min and 1.8 l/m²/min in the moderate hypothermic and hypothermic groups, respectively, when the lowest nasopharyngeal temperatures were reached. Phenylephrine was used as necessary to maintain systemic perfusion pressures at 50–60 mm Hg. Blood collecting in the pericardial cavity was discarded rather than returned to the bypass circuit. Myocardial protection was achieved by the induction of electromechanical arrest with cold, antegrade crystalloid cardioplegia using St Thomas’s I solution and topical cooling using normal saline at 4°C. One litre of cardioplegia was administered initially followed by 300 ml every 30 min of cross-clamping or earlier whenever electrical activity was seen. Distal anastomoses were completed during a single period of aortic cross clamping. Proximal anastomoses were completed on the beating heart using an aortic partial occlusion clamp. Rewarming in the hypothermic and moderately hypothermic groups was commenced at the completion of all distal anastomoses. All patients were rewarmed with a temperature difference of 8°C at the level of the heat exchanger between the blood and the re-warming fluid, and CPB was discontinued only after the patient was fully rewarmed to 37°C. Autologous blood predonated after anaesthetic induction was used for volume replacement and blood remaining in the circuit was reinfused to the patient via a 40μ filter (SQ40S, Pall Europe Ltd., Portsmouth, UK). At the end of the operation, patients were transferred to the cardiac intensive care unit where they were allowed to wake up when haemodynamically stable and blood loss from the chest drains was less than 100 ml/h. When fully warmed up an arterial oxygen tension, on an FiO_2 of 60%, greater than 80 mm Hg, patients were extubated.

The study protocol was approved by the United Bristol Healthcare Trust Ethics Committee and informed consent was obtained from all patients.

2.1. Specimen collection

Samples of blood (10–15 ml) were collected in bottles containing EDTA and placed immediately under ice. Each sample was then centrifuged at 1500 × g for 10 min and the serum was then collected into small Eppendorff vials and frozen to −70°C for later batch analysis. The haematocrit was recorded in order to allow correction for haemodilution. This procedure was undertaken within 15 min of sample collection. Samples were collected preoperatively, 5 min after heparin administration, 30 min after commencement of cardiopulmonary bypass, at the end of cardiopulmonary bypass, 5 min after protamine administration, and 4, 12, and 24 h postoperatively.

2.2. Quantification of inflammatory activity

Neutrophil activity was assessed by measuring concentrations of neutrophil elastase in the serum using an Enzyme Linked Immunosorbent assay (ELISA, Quantime, UK). Complement activation was quantified by measuring the production of C3d a fragment of C3 produced during its conversion to C3a (double decker rocket immunoelectro-phoresis). Interleukin 8, a powerful neutrophil chemotactic factor was measured using an ELISA (R and D Systems, Europe Ltd) and finally, samples were placed on a Gilson Gradient High Performance Liquid Chromatography system to allow detection of eluted IgG fraction. This represents the oxidised form of a protein marker for free radical production, which is then quantified using combined ultraviolet and fluorescence spectroscopy (Beckman System Gold HPLC). Concentrations of the various markers were corrected for haematocrit in order to account for the effects of haemodilution.

2.3. Statistical analysis

Results are expressed as mean ± standard deviation
unless otherwise stated. Continuous variables (Table 1), leucocyte counts and area under the concentration–time curves were compared using the Kruskal–Wallis test. A P-value of 0.05 or less was considered statistically significant. Post-hoc comparisons were performed using single Mann–Whitney tests with Bonferroni-correction. Nominal variables were compared using Chi-squared analysis.

3. Results

Patients were similar with respect to major preoperative and intra-operative variables (Table 1). Although the cardiopulmonary bypass and aortic cross clamp times were longer in the moderately hypothermic groups, this was not statistically significant.

The leukocyte count was increased 24 h postoperatively in all three groups but there was no observed relationship with perfusion temperature (Fig. 1). The time curves (mean ± SE) are shown in Fig. 1(2–5). Neutrophil elastase concentration increased markedly as early as 30 min after the onset of cardiopulmonary bypass, peaked 5 min after protamine administration, and then declined steadily with time (Fig. 2). There was no statistically significant difference between the groups. A similar finding was apparent for C3d release (Fig. 3). Interleukin 8 concentrations also demonstrated a considerable increase related to cardiopulmonary bypass, but there was a rapid decline in interleukin 8 concentrations in the normothermic group in the postoperative period (Fig. 4). Eluted IgG fraction showed a much earlier peak concentration than the other markers, occurring within 30 min of the start of cardiopulmonary bypass (Fig. 5). Levels reached a plateau, before declining soon after the end of bypass and remained higher than preoperative values at 24 h. Again, there was no difference between the three groups. Cumulative marker release was calculated from areas under the concentration–time curves (Table 2). There were no statistically significant differences in cumulative release between groups for all of the markers assayed.

4. Discussion

The results clearly demonstrate that cardiopulmonary bypass is associated with increased concentrations of neutrophil elastase, complement (C3d), IL-8, and free radical activity in agreement with the findings of other workers [8,9]. Normothermia may have reasonably been expected to produce an exaggeration of the inflammatory response to bypass because biochemical pathways are optimal at normal body temperature. The results from this study suggest the opposite however. Free radical activity, neutrophil elastase,...
and C3d concentrations were not shown to be different between groups. In addition, IL-8 concentrations were attenuated, rather than exaggerated, in the normothermic group from 4 to 24 h postoperatively compared to both the hypothermic and moderately hypothermic groups.

The literature remains confusing with regard to the effects of perfusion temperature on the activity of the inflammatory response and even less is known about the clinical sequelae of these responses. Inconsistencies in definitions of normothermic bypass (32–37°C) only perpetuate the controversy. Increased complement activation and IL-1 release have been documented in vitro during normothermia [10] and in a clinical study by Menasche and associates [11], normothermic bypass (35–37°C) was associated with significantly elevated levels of IL-1β and TNF compared to hypothermic bypass (28–30°C). The incidence of vasodilatation, presumed to result from the presence of these mediators, was two-fold higher in the normothermic group necessitating increased use of vasopressors [12]. Steroid pre-treatment may prevent the vasodilatation associated with normothermic cardiopulmonary bypass by inhibition of TNF, IL-6 and IL-8 release [13].

Chello and coworkers performed a randomized study of 20 patients undergoing normothermic bypass (active rewarming to 37°C), and found significantly higher C3a, C5a, C5b-9 and neutrophil activation compared to 20 hypothermic (28°C) controls [14]. The protocol used in this study closely matched that in our study. For example, all patients in the normothermic groups were actively rewarmed to 37°C during bypass. However, Chello [14] used warm blood cardioplegia in the normothermic groups while in our study, cold crystalloid cardioplegia was used in all patients. Nevertheless, the results may not be so apparently conflicting from the present study. Our assessment of complement activity was based upon the concentrations of C3d, a fragment produced by activation of C3 to C3a, and not on levels of C3a, C5a and C5b-9. The influence of perfusion temperature on neutrophil elastase activity remains unclear. In agreement with the present study, Ohata [15] have demonstrated an attenuation of neutrophil elastase 12 h following warm systemic perfusion (34°C). However, other workers have observed lower activity following hypothermic bypass [14,16]. Ohata also found an attenuation of IL-8 concentrations after warm bypass.

Table 2
Cumulative release of mediators

<table>
<thead>
<tr>
<th>Mediator</th>
<th>28°C</th>
<th>32°C</th>
<th>37°C</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil elastase</td>
<td>3172 ± 1038*</td>
<td>3476 ± 744</td>
<td>3179 ± 433</td>
<td>0.49</td>
</tr>
<tr>
<td>C3d</td>
<td>1123 ± 319</td>
<td>1027 ± 208</td>
<td>1262 ± 382</td>
<td>0.28</td>
</tr>
<tr>
<td>IL-8</td>
<td>1154 ± 326</td>
<td>1178 ± 388</td>
<td>889 ± 284</td>
<td>0.09</td>
</tr>
<tr>
<td>IgG fraction</td>
<td>30 ± 6</td>
<td>26 ± 3</td>
<td>27 ± 3</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* Values are mean ± standard deviation.
[15]. IL-8 is a potent neutrophil chemotactic [4] and activating factor [17]. It is released during reperfusion of the ischaemic myocardium [5] and following hypothermic [18] and normothermic cardiopulmonary bypass [9,19]. IL-8 (and TNF-alpha and IL-6) concentrations have been shown to be much higher in patients undergoing heart transplantation, in whom the duration of myocardial ischaemia was much longer than in those undergoing coronary revascularisation [20]. There is evidence linking this cytokine to the pathogenesis of reperfusion injury [21], the postperfusion syndrome [22] and the adult respiratory distress syndrome [4]. While all of the studies mentioned above have provided some insight into the effects of normothermic bypass upon the inflammatory response, the practical significance with regard to end organ dysfunction still requires further evaluation. Of interest would be the effects of normothermic bypass on the systemic response in higher risk patients and those enduring long aortic cross clamp and cardiopulmonary bypass times. The effects of temperature on leukocyte-endothelial interactions needs further elucidation. Neutrophil adhesion molecules CD11a, L-selectin have been measured in warm (32±33°C) and cold (28°C) groups and there is evidence to suggest delayed but inevitable adhesion in the hypothermic patients [23]. P-selectin and E-selectin concentrations were no different in warm (32–33°C) and cold (27–28°C) groups [16].

The concept that normothermia may be associated with an exaggerated inflammatory response to bypass was therefore not demonstrated in this study. One possible reason may be that clearance of these markers may also have been more rapid at higher temperatures, although it is recognised that only circulating mediators can participate in end organ injury.

In conclusion, normothermic systemic perfusion was not shown to produce a more profound inflammatory response compared to hypothermic and moderately hypothermic techniques.

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References


