Cardiopulmonary bypass tubes and prime solutions stimulate neutrophil adhesion molecules

Magdi H. El Habbal *, Linda J. Smith, Martin J. Elliott, Stephan Strobel

Postgraduate Medical Education and Cardiothoracic Unit, Institute of Child Health and The Great Ormond Street Hospital for Children NHS Trust, London WCIN 3IH, UK

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Abstract

Objective: To evaluate effects of the material of the cardiopulmonary bypass (CPB) tubes (polyvinyl chloride, PVC) and prime solutions on expression of neutrophil adhesion molecule CD11b and L-selectin. Methods: We carried out a series of experiments using donor blood from 30 healthy adult human volunteers. In all experiments, neutrophil cell surface expressions of CD11b and L-selectin were assayed immediately and serially up to 2 hours, using immune-fluorescence techniques and flow cytometry. Study 1: Effects of PVC were compared with glass and polystyrene (n = 5). Study 2: Blood was mixed with Plasma-lyte (P) (prime solution), Hartman solutions, albumin or not altered (control), n = 5. Study 3: The effects of changing pH of the P (control, neutralised and acidic solution, n = 5) were examined. Study 4: Haemodilution (undiluted, 1:1, 1:2, and 1:3, vol/vol, prime to blood, n = 5) was carried out using P and the subsequent changes in expressions of the adhesion molecules were analysed. Study 5: The combined effect of PVC and P was assessed (n = 5). Study 6: We evaluated the effect of increasing plasma water by adding sterile water to whole blood and compared it with control (n = 5). Results: Study 1: PVC, similar to glass, caused more up-regulation of CD11b and down-regulation of L-selectin than polystyrene (238 and 162% vs. 68 increase of CD11b, P < 0.001; 89 and 95% vs. 16% decrease of L-selectin, P < 0.001). Study 2: P and Hartman solutions caused more up-regulation of CD11b and down-regulation of L-selectin compared to albumin and control (166 and 188% vs. 26 and 44% increase of CD11b, P < 0.01; 19 and 26% vs. 10 and 6% decrease of L-selectin, P < 0.01, respectively). Study 3: Haemodilution had no effect on these molecules. Study 4: The mean of the difference between the acidic and neutral solution was 208% increase of CD11b and 30% decrease of L-selectin, P < 0.05. Study 5: The combined effect of mixing blood with P and exposure to PVC caused marked up-regulation of CD11b (336% increase, P < 0.01) and down-regulation of L-selectin (79% decrease, P < 0.05). Study 6: Water for injection caused marked up-regulation of CD11b and down-regulation of L-selectin. Conclusions: Mixing blood with acidic prime solution and/or exposing it to PVC tubes causes up-regulation of neutrophil adhesion molecule CD11b and down-regulation of L-selectin. Neutralisation of the prime solution reduces the extent of neutrophil activation, whereas haemodilution has no effect. Increasing plasma water is stimulating to the neutrophil. Modulation of prime solutions and the material of CPB tubes may reduce neutrophil activation which may reduce patient morbidity.

Keywords: Neutrophils; Cardiopulmonary bypass; Adhesion molecules; Prime solution

1. Introduction

Neutrophil activation and the release of its proteolytic mediators have been proposed to contribute to leakage of capillaries and a significant increase in extravascular fluid following cardiopulmonary bypass [1–4]. We have previously documented that up-regulation of CD11b and down-regulation of L-selectin occur in response to cardiopulmonary bypass (CPB) in children [5,6]. After stimulation of the neutrophil with chemoattractants, L-selectin is rapidly shed from the cell surface. Rapid removal of L-selectin from the cell surface may be required for detachment of the neutrophil from the endothelium to allow its migration into tissues [7,8]. The increase in CD11b expression is an important mechanism for transendothelial

* Corresponding author. Tel. +44 171 405-9200, ext. 5934; Fax +44 171 813-8291.

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migration of the neutrophil and induction of vascular injury [7–11]. Neutrophil adherence to endothelium has been shown to control capillary permeability [12]. In adult patients, the effects may not be clinically evident; in young children, however, they are more marked and may be severe enough to cause significant postoperative morbidity and sometimes mortality.

The mechanisms whereby CPB causes inflammatory responses are not well understood. Preparations for CPB include exposing patients’ blood to an artificial surface (polyvinyl chloride), mixing it with a balanced salt solution (prime solution), neutralising the mixture by adding sodium hydrogen carbonate and preventing clotting of blood by the use of heparin [13–15]. These factors, separately or collectively, may account for activation of the neutrophil. In a previous study, we demonstrated that there is an indirect relationship between serum heparin concentration and up-regulation of neutrophil adhesion molecule CD11b and down-regulation of L-selectin [15]. In this study, we carried out a series of experiments using donor blood, examining the effect of CPB artificial surface (polyvinyl chloride, PVC), prime solution (type and pH) and haemodilution on the expression of neutrophil adhesion molecules (which are markers of neutrophil activation). Neutrophil expressions of CD11b and L-selectin were assayed using an immunofluorescence technique and flow cytometry.

2. Methods

2.1. Study subjects

Venous blood samples were obtained, under aseptic conditions, from 30 (collectively) healthy adult volunteers. Their ages ranged between 23 and 38 years. At the time of the study, they had a negative history for inflammatory and/or immunological disease and were not receiving any drug treatment.

2.2. Protocol

The blood sample was placed immediately in containers (containing ethylenediaminetetraacetic acid [EDTA], 1.5 mg/ml of blood, Sigma Chemical Co., MO, USA, No. ED252C). Tubes were shaken (gently) to enable adequate mixing of blood with the medium. Neutrophil cell surface expressions of CD11b and L-selectin were assayed immediately (time 0) and at 30, 60 and 120 min. All experiments were carried out at 37°C in a water bath. Based on the findings in Study 1, experiments 2, 3, 4 and 6 were carried out in polystyrene tubes.

2.3. Methods

Study 1: The effect of exposing blood to the artificial surface was examined. Blood (1 ml of blood, n = 5) was placed in containers (similar in shape) made of polyvinyl chloride (CPB tubes), glass or polystyrene. Laboratory test tubes are commonly made of polystyrene or glass. Neutrophil expressions of CD11b and L-selectin according to the protocol.

Study 2: We tested the effect of the Plasma-lyte™ (Travenol Ltd, Thetford, UK) and Hartman solutions (100 µl/ml blood) which are commonly used in the preparation for CPB and compared it with albumin (4.5% human albumin, colloid, 100 µl/ml blood) and unaltered blood (n = 5).

Study 3: To examine the effect of haemodilution, blood was mixed with unaltered (pH 5.6) Plasma-lyte™ (in ratios of undiluted (0:1), 1:1, 1:2 and 1:3, vol/vol, prime to blood. The degrees of haemodilution was chosen to simulate those used clinically during open heart surgery in children.

Study 4: Effect of altering pH of the prime solution on expression of neutrophil CD11b and L-selectin was evaluated by mixing blood with Plasma-lyte™ (unaltered solution, pH 5.6), neutralised Plasma-lyte™ (solution (pH 7.3, by adding sodium hydrogen carbonate) compared to control blood (n = 5).

Study 5: We evaluated the combined effect of Plasma-lyte™ solution and PVC on neutrophil expression of CD11b and L-selectin. Donor blood was mixed with Plasma-lyte™ solution (1.25:1, vol/vol). The Plasma-lyte™ solution was neutralised (pH 7.3) using sodium hydrogen carbonate. The mixture was placed in polyvinyl chloride tubes (n = 5). The degree of dilution is similar to that which we use clinically. Samples were examined for the expression of neutrophil adhesion molecules CD11b and L-selectin.

Study 6: We evaluated the effect of increasing plasma water by adding 100 µl of water for injection to 1 ml of blood and compared it with controls (unaltered blood, n = 5). Neutrophil expressions of CD11b and L-selectin were assayed according to the protocol.

2.4. Immunofluorescence technique

The neutrophil expression of CD11b and L-selectin was assessed using an immunofluorescence technique and flow cytometry [16] Monoclonal antibodies for CD11b (anti-CD11b, FITC-labelled, mouse IgM, Cymbus Bioscience, UK, No. CBL145), L-selectin (TQ1-RDI, mouse IgG1, phycoerythrin-labelled, Coulter Immunology, Hialeah, FL, USA, No. 6602944), and a negative control (mouse monoclonal IgG1, Kappa, FITC-labelled, Dakopatts, Denmark, No. 927MKB) were used. Aliquots of 25 µl of blood were placed in a Falcon FACS tube (Becton Dickinson, NJ, USA, No. 2052) to which the monoclonal antibodies were added (final concentration of 10%) and incubated for 10 min at room temperature. Red blood cells were lysed by using FACS lysing solution (FACSc® Brand Lysing Solution, Becton Dickinson, San Jose, CA, USA, No. 92-0002)
for 10 min. The neutrophils were centrifuged at 100 × g (4 min) and the supernatant was decanted. Cells were then washed once in 3 ml of HBSS solution (Hanks’ Balanced Salt, without calcium, magnesium, or phenol red. Gibco, UK, No. 041-04175M) with 0.1% sodium azide (Sigma Chemical Co., UK). They were suspended in 1% buffered formaldehyde (100 ml sterile distilled water + 2 g D-glucose [BDH Co., UK, No. C6H12O6] + 1 ml formaldehyde [BDH Co., UK] + 1 tablet of phosphate-buffered saline [Dulbecco ‘A’, UK, No. BR14a]) and stored in the dark at 4°C. At the end of the experiment all samples were analysed at the same sitting using a flow cytometer (FACSScan, Becton Dickinson, San Jose, USA) and the results were expressed in relative fluorescence units. The neutrophils were identified according to their forward and side scatter (based on cell size) as previously described [6].

2.5. Statistical analysis

Data are expressed as median and range between brackets and dotted as mean ± standard error. The Friedman test was used for statistical analysis of time-dependent parameter changes within each group. Because of non-normal distribution of the data, a Mann-Whitney test was applied to assess the difference between samples which were taken simultaneously. Values of $P < 0.05$ were considered statistically significant.

3. Results

All study subjects had normal white cells and neutrophil counts. The range of total white cell count was $5.3–6.2 \times 10^9/\text{l}$ with a median of $5.6 \times 10^9/\text{l}$. The neutrophils represented 45–58% of the total count with a median of 51%.

3.1. Study 1 (Fig. 1a, b)

Marked up-regulation of CD11b and down-regulation of L-selectin occurred in response to exposing donor blood to polyvinyl chloride (CPB tubes) or glass compared with polystyrene (laboratory test tubes).

3.2. Study 2 (Fig. 2a, b)

Mixing blood with Plasma-lyte$^{(148)}$, or Hartman solution, caused more up-regulation of CD11b and down-regu-
lution of L-selectin than albumin or control. The medians of up-regulation of CD11b were 208 (88–393) for Plasma-lyte™(148) and 235 (106–300) for Hartman vs. 33 (21–125) for albumin and 55.8 (11–100) for the control ($P < 0.01$). The medians of down-regulation of L-selectin were 45 (43–167) for Plasma-lyte™(148) and 61 (12–156) for Hartman vs. 24 (−15–98) for albumin and 15.8 (−45–90) control ($P < 0.01$).

3.3. Study 3 (Fig. 3a,b)

Similar to Study 1, up-regulation of CD11b and down-regulation of L-selectin occurred upon mixing with Plasma-lyte™(148). However, the changes were unrelated to the degree of haemodilution. Medians and ranges of up-regulation of CD11b were 162 (71–193) for 1:1 dilution, 122 (102–131) for 1:2 dilution, 186 (95–195) for 1:3 dilution ($P > 0.1$). The medians of down-regulations of L-selectin were 78 (68–145) for 1:1 dilution, 94 (84–123) for 1:2 dilution and 83 (70–127) for 1:3 dilution ($P > 0.1$).

3.4. Study 4 (Fig. 4a,b)

Increasing pH of the prime solution reduced the extent of activation of neutrophil adhesion molecules. The median of difference between the acidic and neutral solution was 112 (52–170) for CD11b and 62.3 (4–120) for L-selectin, $P < 0.05$.

3.5. Study 5 (Fig. 5 a,b)

Mixing blood with Plasma-lyte™(148) and placing it in PVC tubes caused more up-regulation of CD11b than each factor separately (Study 1 and Study 2), $P < 0.01$. Down-regulation of L-selectin was similar to that of PVC (Study 1), but more than mixing with Plasma-lyte™(148) (Study 2), $P < 0.05$. The medians of up-regulation of CD11b and down-regulation of L-selectin were 525 (216–827) and 212 (126–271), respectively.

3.6. Study 6 (Fig. 6a,b)

Adding sterile water to blood caused marked up-regulation of CD11b and down-regulation of L-selectin compared with controls.

4. Discussion

These studies demonstrate that up-regulation of neutrophil adhesion molecule CD11b and down-regulation of
L-selectin occur in response to mixing blood with crystalloid solutions (prime solution of CPB) and/or exposure to polyvinyl chloride (CPB tubes). Previous studies showed that heparin induced up-regulation of neutrophil adhesion molecule CD11b, down-regulation of L-selectin and the release of inflammatory mediator [15]. These responses were shown previously to be modulated by hypothermia and rewarming [6]. Therefore, CPB induced neutrophil activation by various factors. Exposing human blood to artificial surfaces is known to cause stimulation of the neutrophil [16]. It seems that the extent of neutrophil activation may depend on the type of material of the artificial surface. There was more up-regulation of CD11b and down-regulation of L-selectin upon exposure to polyvinyl chloride and glass than to polystyrene. Polyvinyl chloride is routinely used for the making of CPB tubes because of its transparency and flexibility. The transparency enables visualisation of any air leakage into the system and its flexibility allows manipulation during surgery. It seems that adaptation of polystyrene may be more appropriate to use. Polystyrene which is used for the making of laboratory test tubes was less stimulant to neutrophil adhesion molecules than that of polyvinyl chloride. However, polystyrene is a stiff substance which may not be practical to use. Silicon (which is considered to be less stimulant to leukocytes) tubes are opaque, which may limit detection of air leaks into the CPB [17]. It was thought that the activation of leukocytes and the inflammatory response initiated during the CPB can be inhibited by administering heparin so as to reduce contact between blood cells and the artificial surface of the CPB (heparin coating of CPB) [18]. Interestingly, heparin coating of circuits did not reduce C3a generation but reduced its increase after protamine administration which is known to activate the complement cascade through the classic pathway [19].

Most if not all paediatric CPB’s are carried out under hypothermic conditions. Hypothermia causes vasoconstriction. Therefore, crystalloid is used in CPB to reduce blood viscosity which is thought to be essential for maintaining circulation into small blood vessels during hypothermia [14]. Mixing blood with crystalloids may cause an increase in plasma water content [20]. It seems that mixing blood with crystalloids causes marked activation of the neutrophil. Mixing blood with Plasma-lyte"(148) and Hartman solutions caused marked up-regulation of CD11b and down-regulation of L-selectin compared with albumin and

Fig. 5. Mixing blood with Plasma-lyte" solution and placing it in polyvinyl chloride tubes caused up-regulation of CD11b and down-regulation of L-selectin to reach levels higher than those observed when blood was mixed with crystalloids but similar to those carried out in polyvinyl chloride tubes.

Fig. 6. Adding water for injection to blood caused up-regulation of neutrophil cell surface expression of CD11b and down-regulation of L-selectin.

**Fig. 5.** Mixing blood with Plasma-lyte" solution and placing it in polyvinyl chloride tubes caused up-regulation of CD11b and down-regulation of L-selectin to reach levels higher than those observed when blood was mixed with crystalloids but similar to those carried out in polyvinyl chloride tubes.

**Fig. 6.** Adding water for injection to blood caused up-regulation of neutrophil cell surface expression of CD11b and down-regulation of L-selectin.
control blood. We tested the effect of increasing water content by adding water for injection to blood obtained from healthy volunteers. This experiment showed marked up-regulation of CD11b and down-regulation of L-selectin. Therefore, reducing total body water using modified ultrafiltration [5,21] (direct haemconcentration) perioperatively and/or air-flow kinetic concepts postoperatively [22] (increase in insensible water loss) may reduce tissue oedema and reduce patient morbidity.

Although both Plasma-lyte®[148] and Hartman solutions caused a similar effect on neutrophil adhesion molecules, differences exist between the two solutions, particularly the presence of lactate buffer in the Hartman solution. However, the pH of both solutions was similar (pH 5.6). It seems that pH alteration may have a significant effect on crystalloid stimulation of neutrophil expression of its adhesion molecules. There were more up-regulation of CD11b and down-regulation of L-selectin with acid plasma-lyte solution than with neutral solution (pH 7.3). Previous studies showed that extracellular acidic pH modulates oxygen-dependent cytotoxic responses mediated by polymorphonuclear leukocytes and monocytes [23].

There was concern that haemodilution was the cause of tissue oedema which was observed in children who undergo open heart surgery. It seems that the degree of haemodilution using crystalloid solution has no relationship to the extent of neutrophil activation. Up-regulation of CD11b and down-regulation of L-selectin occurred upon mixing blood with crystalloid solution at 1:1 (100 μl:1 ml), 1:3, 1:2 and 1:1, volume to volume of crystalloid to blood. However, haemodilution is associated with increasing the water content of plasma, which may, directly, cause tissue oedema irrespective of neutrophil activation.

It appears that exposing blood to the artificial surface of the CPB is more stimulating to the neutrophil than mixing it with crystalloid solutions. There was more up-regulation of CD11b and down-regulation of L-selectin when blood was exposed to PVC than when mixed with Plasma-lyte or Hartman solutions. However, it seems that mixing blood with crystalloids augments the stimulating effect of PVC. The up-regulation of CD11b was higher in the experiments whereby blood was mixed with Plasma-lyte and placed in PVC tubes than that of blood exposed to PVC or mixed with crystalloid solution, separately. Because of the very low level of L-selectin at end of 2 h, the differences between the experiments were insignificant.

Thus, CPB in its current form is non-physiological in as much as it stimulates neutrophil adhesion molecules. Modification of preparations for CPB may reduce neutrophil activation. Alternatively, the use of additional techniques such as modified ultrafiltration and/or advances in postoperative management of patients who undergo open-heart surgery (e.g., using air-flow kinetics) may reduce tissue oedema and patient morbidity.

References

