Impact of xenon anaesthesia in isolated cardiopulmonary bypass on very early leucocyte and platelet activation and clearance: a randomized, controlled study

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Background. Cardiopulmonary bypass (CPB) is associated with leucocyte and platelet activation and also organ dysfunction. Xenon has been found to have organ-protective effects. We therefore investigated the effect of isolated CPB on leucocyte and platelet activation and the efficacy of xenon in inhibiting these changes.

Methods. Isolated CPB was conducted according to strict standardized clinical criteria using blood from healthy volunteers. They were randomized to an air–oxygen mixture (control group) vs xenon–oxygen mixture (xenon group). Blood samples were drawn at 5, 15, 30, 60, and 90 min from commencement of circuits and analysed for haemoglobin concentrations, white cell, neutrophil, monocyte, lymphocyte, and platelet counts. Leucocyte and platelet activation and also complex formation were determined by measuring levels of CD14++ monocytes, CD16+ monocytes, platelet–monocyte complexes, and platelet–neutrophil complexes (PNC). Differences between and within the groups were analysed with Student's t-test.

Results. Biomarker levels were not different between the groups. The data were pooled to identify the effects of isolated bypass. The neutrophils, monocytes, platelets, CD14++ monocytes, and CD16+ monocytes decreased within 5 min of the bypass experiments, whereas the percentage of platelet–CD++ monocyte complexes and PNC increased.

Conclusions. Isolated CPB elicited rapid, substantial leucocyte and platelet activation, and xenon had no impact on inhibiting these changes.

Br J Anaesth 2009; 103: 805–10

Keywords: blood, platelets; blood, neutrophils; heart, cardiopulmonary bypass; immune response, suppression; special drugs, xenon

Accepted for publication: September 10, 2009

Cardiopulmonary bypass (CPB) is associated with an increase in the blood concentration of inflammatory markers.2 3 Organ dysfunction and significant neurological impairment, including stroke in 1.5–3.2% and cognitive impairment in up to 60% of patients, are attributed to a multitude of factors including cellular inflammatory responses.4 5 Cellular indices of inflammation include monocyte and platelet activation and can be detected by measuring leucocyte–platelet complex formation.5 6 Early increases in blood cytokine concentration are reported in stroke cases after CPB.7 Experimental and clinical studies implicate activation of platelets and monocytes in both acute and chronic effects on the coronary and neurovascular circulation.8–10

Xenon, an insoluble, non-metabolized, noble gas, was first described to have anaesthetic properties in 1948. In experimental and preliminary clinical studies, it was shown to have both neuroprotective and cardioprotective effects.11–17 It has also been shown to have a modulating effect on neutrophil adhesion molecule expression
Possible interactions with circulating and endothelial mediators of inflammation and the effect of xenon on leucocyte activation prompted the hypothesis that protective effects might reflect inhibition of platelet and leucocyte activation. In particular, we tested the hypothesis that indices of platelet and leucocyte activation are significantly lower in an isolated model of CPB when xenon is added to the standard gas mixture.

**Methods**

After obtaining Ethical Committee approval, written informed consent to use donated blood was obtained from eight healthy volunteers. The inclusion criteria were set in accordance with UK blood donation guidelines. Further exclusion criteria were operations within the last 6 months and regular medication. The suitable volunteers donated 500 ml of blood. After donation, 3000 units of heparin were added to the blood collection bag, and the sample was divided into two equal aliquots of 250 ml each. The donated blood was immediately circulated in two identical circuits by an experienced perfusionist. The circuits were randomly allocated to be ventilated either with 50% xenon in oxygen (study circuit) or with 50% oxygen in air (control circuit). Study and control circuits were coded to ensure that laboratory analysis on the blood samples was carried out in a blinded fashion. The gases were delivered by roller pumps at 0.25 ml min⁻¹ to each of the membrane oxygenators. Gas mixtures were controlled using oxygen analysers (AX300 Medical Oxygen Analyzer, Teledyne Analytical International, Wantage, UK) on the inspiratory limb. The flow was maintained at 0.5 litre min⁻¹. All parameters were maintained within clinically accepted limits (blood temperature, 37°C; pH, 7.2–7.4; \( P_{O_2} \geq 25 \) kPa; \( P_{CO_2} \), 4.5–5.5 kPa). After 90 min, circulation was terminated and the circuits were discarded.

Blood samples were collected immediately after donation and at 5, 15, 30, 60, and 90 min after starting circulation. Blood was drawn into ethylene diamine tetra acetate tubes and then immediately transferred to Diatube-H Vacutainers (BD, Becton Dickinson, Oxford, UK), containing citrate, theophylline, adenosine, and dipyridamole. All blood samples were analysed within 6 h for red blood cell counts, white blood cell counts (total and differential counts), and platelet counts by ABX Pentra 60 (Horiba ABX Diagnostics, Sheffield, UK).

The antisera used were fluorescein isothiocyanate conjugated mouse IgG1 HLA-DR, phycoerythrin (PE) conjugated mouse IgG1 CD42A, PE-Texas Red (ECD) conjugated mouse IgG1 CD14, and PE-cyanin 5 (PC5) conjugated CD16 (Beckman Coulter, High Wycombe, UK). Fifty microlitres of anticoagulated blood were labelled within 30 min of collection by incubation with optimal concentration of antibodies for 15 min at room temperature, lysed and fixed using Optilyse C, and washed and resuspended in 500 μl of phosphate-buffered saline with 0.5% bovine serum albumin. Samples were analysed using an EPICS-XL flow cytometer, calibrated and standardized with Flow-Check™ and Flow-Set™ beads, with Expo-32 software (Beckman Coulter). Leucocytes were identified using standard light characteristics plotting forward scatter vs side scatter, then platelet–neutrophil complexes (PNCs) identified by the proportion of cells in the neutrophil population expressing the platelet marker CD42A. Monocyte subsets were identified by sequential gating as CD14++ HLA-DR+ CD16– and CD14+ HLA-DR++ CD16+ and platelet–monocyte complexes (PMCs) identified by co-staining with CD42A. Study and control circuits were allocated at random and coded to ensure that laboratory analysis was carried out in a blinded fashion. One of the xenon circuits had to be terminated at 82 min (8 min before the completion time) because of loss of volume, and blood samples were obtained just before stopping the circuit.

**Statistical analysis**

The number of bypass experiments needed was based on a previous similar study in which 10 bypass experiments were needed to show a difference in the inflammatory marker levels (power 90%, \( \alpha \)-value 0.05). As our interim analysis showed no difference between the groups, we stopped the study after eight bypass experiments. The haemoglobin concentration on bypass varied little after the initial haemodilution. Accordingly, cell counts and inflammatory indices for the blood samples from each circuit were normalized for haemodilution using the formula: haemoglobin concentration at baseline divided by sample haemoglobin concentration before subjecting the data to statistical tests. Paired \( t \)-tests were used for statistical comparison between the two groups at discrete time points. We also tested for changes in parameters over time within and between the groups using paired \( t \)-tests. A \( P \)-value of ≤0.05 was regarded as significant. Values are reported as the mean (SEM) unless otherwise stated.

**Results**

The characteristics of the donors and the bypass circuits were comparable. We analysed the measurements after correcting for haemodilution as described earlier.

There was a rapid, significant reduction in leucocyte counts (expressed in \( 10^9 \) litre⁻¹) evident within 5 min of commencing circulation in both the groups which continued during the first 30 min (Fig. 1a). Similarly, there was a very early reduction in neutrophil counts (Fig. 1b) and monocyte counts (Fig. 1c) that continued during the first 30 min in both the groups (\( P<0.05 \)). There was no significant difference in white cell counts, neutrophils, and monocytes between the groups at any time point.
Changes in leucocyte and platelet counts during isolated CPB in the control and the xenon groups. Serial leucocyte (A), neutrophil (B), monocyte (C), and platelet (D) counts (in 10^9 litre^(-1)) are shown plotted against time in minutes in the control and the xenon groups during isolated CPB, with normalization for haemodilution using the formula: haemoglobin concentration at baseline divided by the mean haemoglobin concentration during isolated CPB. Data are mean (SEM). There is no significant difference between the groups for any parameter at any time point, P>0.05. Leucocyte counts (A) vs baseline in controls P=0.05 at 5 min, P<0.02 at 15, 30, 60, 90 min; for xenon at 5 min P=0.002, P<0.001 thereafter; vs 5 min in controls P<0.01 at 15 min, P=0.005 at 30 min, and for xenon P=0.009 and 0.04, respectively. Neutrophil counts (B) vs baseline in controls P=0.034 at 5 min, P<0.05 thereafter, for xenon P=0.005 at 5 min, P<0.005 thereafter; vs 5 min P<0.05 at 30 min in both the groups. Monocyte counts (C) vs baseline in controls P<0.05 at 5 min, P<0.01 thereafter, for xenon P<0.005 at 5 min and thereafter; vs 5 min for both the groups P<0.01 at 30 min and vs 15 min P<0.05 at 30 min. For platelet counts (D) vs baseline in controls P=0.02 at 5 min, P<0.05 at 15 min, no significant difference thereafter, for xenon P<0.001 at 5 min, P<0.01 at 15, 30 min, no significant difference thereafter; vs 5 min for both the groups P<0.001 at 30, 60, 90 min. *P<0.05 and **P<0.005 vs baseline.

Serial platelet counts (expressed in 10^9 litre^(-1)) revealed a bimodal pattern of change in both the groups (Fig. 1b). There was a very early decrease in platelet counts at 5 min in both the groups (P<0.05) followed by recovery at 30 min (P<0.005 vs 5 min for both the groups). Platelet counts continued to increase through the rest of the study period (P<0.001 at 90 vs 5 min for both the groups). There were no significant differences in platelet counts between the groups at any time point.

There were no significant differences in CD14++ and CD16+ monocyte subsets, PMCs, and PNCs between the control and xenon-treated groups for any time point.

As the substantial changes in the different cell counts occurred after initiation of bypass experiments and no significant differences were found between the groups, the data were pooled to determine the effect of isolated bypass (Fig. 2). In comparison with baseline, neutrophil and monocyte counts declined significantly during the bypass experiments up to 30 min. In contrast, platelet counts decreased at 5 min to 46.6% (14.2) of baseline value (P<0.0001) followed by a steady increase during bypass.
experiment. The increase in platelet count after 5 min was significant at 15, 30, 60, and 90 min.

CD14++ monocyte counts, CD16+ monocyte counts, and the total number of PMCs showed a very early decrease and in comparison with baseline, they were statistically significant at the various time points measured (Fig. 3). There was no change in the proportion of CD16+ monocytes complexed with platelets. In contrast, the proportion of CD14++ monocytes complexed with platelets increased significantly in 15 min, and remained higher than baseline values. The proportion of neutrophils complexed with platelets remained higher after 5 min, and the increase was significant at 15 min.

**Discussion**

We found an early substantial clearance of leucocytes from the circulation that principally affects neutrophils and monocytes and a bimodal pattern of platelet clearance during isolated CPB. One potential explanation for the observed leucocyte and platelet clearance is the platelet and leucocyte activation with complex formation which is known to occur within a few minutes of stimulation. The bimodal or mixed pattern observed is consistent with the dynamic nature of PNC and PMC formation, since both platelet—leucocyte and platelet—endothelial complexing are known to be reversible. A strategy of early sampling combined with sensitive assays highlighted these very early changes.

We investigated PNCs and PMCs since they are sensitive markers of leucocyte and platelet activation, and also being implicated in the inflammatory response during CPB and in cardiovascular complications. In contrast, levels of CD11/CD18 expression show a limited correlation with leucocyte adhesion and clinical complications since integrin function is critically related to conformational changes. PMC formation is also associated with activation of NF-kB and induction of cytokine mRNA, providing a potential pathway for late inflammatory complications of CPB. The CD14++ and CD16+ monocyte subsets we measured vary in their expression of chemokine receptors and adhesion molecules and also their migratory and differentiation properties, but both are implicated in cardiovascular disease and production of proinflammatory cytokines. Taken together with our strategy of early sampling, our indices are potentially more discriminating, therefore, than measurement of cytokine concentration and adhesion molecule expression.

Despite early sampling and sensitive assays of clinical relevant indices, we were unable to identify significant differences between the control and xenon-treated groups. Xenon has been shown to decrease the neutrophil adhesion molecule expression in vitro, but other studies report little effect on platelet activation or cytokine production. In our study, xenon failed to have any significant effect in inhibiting the cellular markers of inflammation.

As there were no differences between the groups, we pooled the data to determine the magnitude of activation of cells during bypass. We found evidence of significant changes in neutrophil, monocyte, and platelet counts within the first 5 min of CPB. Our detailed investigations indicated clearance of both CD14++ and CD16+ monocyte subsets which was significant after 15 min of isolated bypass. These very early changes occurring within 5 min of CPB have not been shown by previous studies.
These changes were associated with an increase in the proportion of monocytes and neutrophils complexed with platelets after 15 min by almost two-thirds and one-third, respectively. This is probably an underestimate of PMC and PNC formation, since the total number of PMCs had decreased by almost 20% after 15 min of CPB. The substantial reduction in platelet count is consistent with the evidence that each monocyte binds several platelets when PMCs are formed.8 9 Recovery of platelet counts during the bypass experiment is consistent with some dissociation of leucocyte–platelet complex formation with re-emergence of activated platelets and possibly some activated leucocytes into the circulation.8 These findings are consistent with the cellular inflammatory response seen during CPB.2

Our study used isolated CPB as a surrogate for CPB in order to test our hypothesis. Simulated or isolated CPB is a well-established technique for investigating potential agents for therapeutic inhibition of the cellular inflammatory response during CPB.22 28 29 Limitations of this technique include the lower flow rate, smaller prime volume, and higher dilution than in clinical practice.

Our findings, together with earlier studies, highlight the importance of platelet activation in leucocyte–platelet complex formation, leucocyte clearance, and potentially delayed, cytokine-mediated effects via NF-kB activation.25 This is likely to be clinically relevant, since genetic variants in P-Selectin that influence platelet activation correlate with susceptibility to cognitive decline after cardiac surgery involving CPB.30 Taken together, these data suggest that it may be necessary to target multiple pathways such as complement C5a and P-Selectin in order to affect outcome.28

We conclude that isolated CPB is associated with very early changes in the cellular mediators of inflammation that are not inhibited by xenon. We suggest that isolated CPB is a safe and established technique, and early and frequent sampling combined with sensitive assays of clinical relevance can test for efficacy of therapies to inhibit the responses induced by CPB.

Acknowledgements
We acknowledge the Department of Clinical Perfusion in Papworth Hospital NHS Foundation Trust for their assistance in conducting the study. We also acknowledge Samantha Buckenham MSc, Biomedical Scientist, Immunology Laboratory, Department of Pathology, Papworth Hospital NHS Foundation Trust, Cambridge, UK, for expert technical assistance and Carl Atkinson, PhD, Research Assistant Professor, Medical University of South Carolina, Department of Microbiology and Immunology, Charleston, SC, USA, for scientific discussions.

Funding
This study was kindly supported by Linde Health Care with a financial grant.

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