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Mini bypass and pro-inflammatory leukocyte activation: a randomised controlled trial

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ABSTRACT

Background: Coronary artery bypass grafting (CABG) with conventional cardiopulmonary bypass (CPB) induces systemic inflammation. Miniaturized CPB may attenuate systemic inflammatory activation. The intracellular signalling pathways promoting inflammation in cardiac surgery and the relative effects of CPB on these processes are uncertain. In this study, induction of reactive oxygen species (ROS) and activation of NF-κB, p38 mitogen activated protein kinase (MAPK) within leukocytes and leukocyte accumulation in cantharidin-induced blisters was compared in patients exposed to miniaturized (mCPB) or conventional CPB (cCPB).

Methods: Patients undergoing CABG were randomised to receive either cCPB (n=13) or mCPB (n=13). Blood samples were collected pre-operatively and five times after initiating CPB (up to 5 hours) and analysed by flow cytometry for intracellular markers of activation (ROS, p38-MAPK and NF-κB phosphorylation).

Results: ROS in lymphocytes were elevated in cCPB compared to mCPB (p<0.01) whereas ROS in granulocytes and monocytes were similar between groups. Following initiation of CPB, p38-MAPK was higher in patients receiving cCPB compared to mCPB (p<0.05). NF-κB phosphorylation in leukocyte subsets was similar in patients exposed to cCPB or mCPB. Leukocyte accumulation in cantharidin-induced blisters, white cell counts and serum C-reactive protein were enhanced in response to cardiac surgery, but no differences were observed between mCPB or cCPB groups. Post-operative serum creatinine levels were reduced in the mCPB group compared to cCPB (p<0.05).

Conclusion: Both p38-MAPK activation and ROS were attenuated with the use of mCPB compared to cCPB, providing a potential mechanism for reduced inflammation in association with CPB miniaturisation.

INTRODUCTION

Coronary artery bypass grafting (CABG) is frequently performed with cardiopulmonary bypass (CPB). However, CPB is known to evoke a systemic inflammatory response[1]. Miniaturised CPB has been developed to attenuate systemic inflammation by optimising the components of the CPB circuit. Optimisation may be achieved through: reducing haemodilution by minimising the priming volume of the circuit; eliminating the blood-air-interface by avoiding the use of a venous reservoir; replacing the use of cardiotomy suction with a cell-salvage device; and coating the circuit with a biocompatible coating (e.g. phosphorylcholine), which would be expected to reduce contact activation. In previous studies, mCPB has been shown to reduce blood loss and transfusion requirements[2, 3] and to lessen renal, myocardial and intestinal injury[2, 4, 5]. The inflammatory response has also been shown to be attenuated with the use of mCPB as demonstrated by reduced cytokine release and neutrophil activation[6-8]. Despite studies demonstrating that cardiac surgery with the use of CPB induces systemic inflammation, the pro-inflammatory signalling pathways activated by CPB have not been precisely defined. Moreover, the cell-signalling mechanisms underlying the inflammatory effects of CPB miniaturisation have not been characterised at a molecular level. To address this question, a clinical trial to determine the kinetics of inflammatory signalling pathway activation was performed in patients undergoing CABG with conventional CPB (cCPB) or miniaturised CPB (mCPB). The primary hypothesis was that, compared to cCPB, the mCPB system would reduce ROS / pro-inflammatory activation *within* leukocytes and attenuate systemic inflammation measured in blood samples. A secondary hypothesis was that the use of the mCPB system would reduce the degree of neutrophil and monocyte margination from within the circulation into fluid in the extravascular tissue compartment, harvested from cantharidin blisters.

MATERIALS AND METHODS

Study design

A single centre, parallel-group RCT was performed (ISRCTN30610605). A favourable local ethics opinion was obtained from the 'National Research Ethics Service, South West London' Research Ethics Committee (REC reference 08/H0708/67). Informed written consent was obtained from research participants.

Participants

Patients undergoing primary isolated CABG by a single surgeon were considered. Patients <18 years of age; emergency surgery; ejection fraction <30%; recent cerebro-vascular accident; >75% carotid artery stenosis; renal impairment (serum creatinine >177 μmol/L); pre-existing coagulopathy, pre-existing liver dysfunction or recent (within 5 days) use of antiplatelet agents (aspirin/clopidogrel) were excluded.

Intervention and comparator

Anaesthesia was standardized for all patients. Thiopentone (1–3mg/kg) was used for induction with 3–5mg/kg fentanyl, and volatile agents were delivered in 50% air–O_2 for maintenance. Propofol (3mg/kg/h) was infused during CPB and neuromuscular blockade was achieved using 0.1–0.15mg/kg pancuronium. Intravenous heparin (3 mg/kg) was used to maintain an activated clotting time >480s.

Operations using cCPB or mCPB involved aortic cannulation, two-stage venous cannulation and moderate hypothermia (32°C) with parallel blood cell salvage (Electa, Sorin group, Milan, Italy). cCPB was conducted as described[9]. mCPB was conducted using the Sorin Dideco Extracorporeal Circulation Optimized (ECCO) system as described [10]. Coronary artery anastomoses were constructed using intermittent cross-clamp fibrillation (ICCF) as previously described[11].

Outcome measures

Primary outcome was intracellular ROS activation in granulocytes as these were considered the most rapid markers of pro-inflammatory signalling within the cell lineage first activated in acute inflammation[12].

Secondary outcomes were ROS activation in monocytes and lymphocytes; activation of pro-inflammatory p38 mitogen-activated protein kinase (MAPK) which promotes inflammation by activating downstream AP-1 superfamily transcription factors [13] and activation of NF-κB transcription factor which activates multiple pro-inflammatory genes [14] measured as the phosphorylated p65 subunit. Secondary outcomes also included leukocyte counts in cantharidin-induced blisters, white cell counts, serum CRP and creatinine levels. Sampling times are summarised in Figure 1.

Assays of intracellular ROS, p38-MAPK activation and NF-κB activation

ROS were measured by combining (1:10) diluted blood with 3'-(p-aminophenyl) fluorescein (APF; Molecular Probes, Eugene, OR, USA)for 30 min at 37°C prior to erythrocyte lysis. In parallel, leukocytes were fixed with phosphatase buffers and isolated using erythrocyte lysis and centrifugation using manufacturer-published BD phosflow methods prior to incubation with phycoerythrin (PE)-Cy7-conjugated antibodies that recognise phosphorylated p38-MAPK and PE-conjugated antibodies that recognise Ser529 phosphorylated NF-κB (BD Biosciences, San Jose, CA, USA). Fluorescence was quantified in granulocytes, monocytes or lymphocytes (forward and side scatter) using flow cytometry and *Summit 4.3* software.

Quantitation of leukocyte migration

Cantharidin was applied to the forearm to generate a blister, and leukocyte sub-populations in blister fluid were identified and counted using the Diff-Quick kit (Polysciences Inc , Warrington, PA, USA) and microscopy as described [9].

Sample size

Although the primary outcome was intracellular ROS activation in leukocytes, the target sample size was calculated on the basis of counts of post-operative leukocytes in cantharidin blisters, adjusted for baseline levels. We did this because the latter measurements were only made before and after the intervention (rather than having multiple post-operative measurements). Hence, the trial had less statistical power for this outcome than outcomes measured in blood samples. Based on a previous study[9], we aimed for a sample size of 13 patients per group. We calculated that this sample size would have 95% power to detect a difference in means of 1.448×10^5 cells between groups, based on following assumptions: common standard deviation=1×10^5 with a 0.05 two-sided significance level. A power calculation for signalling outcomes (relating to ROS, p38 and p65/NF-κB) was not carried out in advance because the correlations between pre- and post-, and between longitudinal post-intervention measurements were unknown.

Randomisation

Participants were randomised by the Imperial College Trial Unit staff not otherwise involved in the study. Random allocations were blocked and generated by computer. The random allocations were concealed at the time of recruitment. Randomisation was carried out by one of the investigators after the patient's eligibility had been checked and written informed consent obtained. Participants were blinded to the allocation.

Statistical methods

Data were analysed according to a pre-specified statistical analysis plan (SAP). Final analyses were performed after all subjects completed the study and after the database was locked. Continuously scaled outcomes are described as means and SD for each treatment group, at all time points if measured more than once. A natural logarithm transformation was applied if outcome data were positively skewed, in which case findings are reported on the natural logarithm scale.

The effects of mCPB compared to cCPB were estimated by fitting mixed regression models (using STATA v.11.2) to take into account the repeated measurements. The following predictors were included: main effect of time, fitted categorically (baseline to 4 days after the operation depending on the measurement schedule for the variable) and main effect of the intervention (mCPB vs.cCPB). Interactions between treatment group and time were also fitted and, if statistically significant at the 5% level, differences between groups with 95% confidence intervals (CI) are described for each time point separately; otherwise an overall treatment difference (with 95% CI) is reported. All data analyses were performed on an intention-to-treat basis irrespective of the final actual treatment modality received.

RESULTS

Patient recruitment

The flow of participants through the trial is described in the CONSORT diagram (Figure 2). Fifty patients undergoing CABG with CPB were considered for inclusion into the study. 7 declined to take part, 17 were

ineligible. 26 patients were randomised to mCPB (n=13) or cCPB (n=13). All patients allocated to cCPB had surgery performed as allocated. One participant allocated to mCPB underwent surgery with cCPB because the perfusionist was not fully trained to use mCPB.

Baseline characteristics and operative details

Patient characteristics and peri- and post- operative data are summarised in Table 1 and Table 2.

Primary and secondary outcomes

ROS activation

Distributions for ROS, p38 and p65 were positively skewed and the data were transformed into natural logarithms. Effect estimates for the comparison between mCPB and cCPB groups are presented in Table 3. ROS induction levels in lymphocytes were higher in patients exposed to cCPB compared to mCPB at 30 min (mean difference= -0.39 log units, 95% CI [-0.69 to -0.09], p=0.01; Table 3). ROS induction levels in granulocytes (the primary outcome) and mononuclear cells showed the same pattern as for ROS levels in lymphocytes but the differences between groups were not statistically significant (Table 3, Figure 3A, Figure 4A).

p38-MAPK and NF-κB activation

Distributions for p38-MAPK and NF-κB p65 were also positively skewed and the data were transformed into natural logarithms. Activation of p38-MAPK peaked at the time of initiation of cCPB (t=0 timepoint) and subsequentlydeclined throughout surgery (Figure 3B, Figure 4B). Activation of p38-MAPK in granulocytes was lower in patients exposed to mCPB compared to cCPB (mean difference= 0.47 log units, 95% CI -0.89 to -0.05, p=0.03; Table 3, Figure 3B, Figure 4B). A reverse pattern was observed in mononuclear cells, with p38-MAPK being enhanced, but not significantly, in patients exposed to mCPB compared to cCPB (Table 3, Figure

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3B, Figure 4B). Activation of NF-κB p65 in granulocytes and mononuclear cells did not differ between groups; levels were reduced in response to cardiac surgery irrespective of group, with the lowest levels were observed at 2h and 5h following the start of CPB (Table 3, Figure 3C, Figure 4C).

Leukocyte migration, white cell counts, haemoglobin, creatinine and C-reactive protein

WCC, Hb, Creatinine and CRP data are summarised in Table 4. Summary statistics for leukocyte counts in cantharidin blisters are given in Table 5. Representative light microscopy images are illustrated in Figure 5. There were no significant differences between mCPB and cCPB in leukocyte migration into skin blisters (Table 3). No differences in white cell counts in peripheral blood or haemoglobin were observed between either CPB groups (Table 3, Figure 6B and D). Serum creatinine levels were lower in the mCPB compared to the cCPB group and there was a significant time by group interaction, (p=0.04) despite the treatment effects at day 1 and day 4 days after the operation being individually non-significant (these estimates were in opposite directions; Table 3 and Figure 6C). CRP levels increased on day 1 and were further enhanced on day 4 following surgery, with no difference between either the mCPB or cCPB groups (Table 3, Figure 6A).

DISCUSSION

Main findings

Coronary revascularisation with mCPB compared to cCPB induced a lower early rise (at 30 minutes) in ROS in lymphocytes; levels of ROS in granulocytes or monocytes changed in a very similar manner but differences between groups were not significant. Activation of p38-MAPK in granulocytes was lower in patients exposed to mCPB compared to cCPB but not in mononuclear cells. There were no differences in NF-κB activation

between the mCPB and cCPB groups. Despite the differing characteristics of p38-MAPK and ROS activation, the total numbers of migrating leukocytes into tissues did not differ between the two groups. There were also attenuated serum creatinine levels in the mCPB group.

Effects of CPB miniaturisation on leukocyte activation

Our institution has developed extensive experience with mCPB, proven to be safe and effective[10]. mCPB has been associated with lower levels of inflammatory cytokines including IL-6[15-17], IL-8[6], TNFα[16], MCP-1[15] and neutrophil elastase [6, 16] suggesting less immune activation compared to conventional systems. In addition, levels of oxidative stress, indicated by elevated malondialdehyde levels, were lower in mCPB patients[18]. Here we compared the effects of CPB systems on pro-inflammatory

signalling molecules. We chose to study the activation of signalling molecules in leukocytes because they

can act as rapid sentinels of the inflammatory response.

p38 -MAPK activation in granulocytes was significantly reduced in patients exposed to mCPB compared to cCPB. By contrast, p38-MAPK actiation in mononuclear cells, ROS induction and NF-κB activity in both granulocytes and mononuclear cells was similar between both groups. We conclude from these data that mCPB had specific inhibitory effects on p38-MAPK activation in granulocytes. Interestingly, mCPB and cCPB were associated with comparable numbers of leukocytes migrating into blisters and equivalent numbers of circulating white blood cells.

What is uncertain is the reactivity of those leukocytes within the tissue compartment. As an adaptive response to injury, further work is required to understand the transition between inflammation, resolution and healing. Our data is in keeping with other clinical studies showing preservation of renal function[2]. Whether this is due to the mCPB intervention is uncertain.

We have shown that coronary surgery with ICCF leads to the activation by phosphorylation of p38-MAPK, an

inflammatory signalling intermediary, in leukocytes and induction of ROS. The ICCF technique is standard

practice in our institution but is used less frequently elsewhere and previously shown to be as effective as

other methods of myocardial protection[19]. We believe our manuscript is the first to report ROS, p38-

MAPK and NF-κB changes in surgery with ICCF. The effects of cardioplegic arrest on pro-inflammatory

leukocyte signalling and different modalities of cardiac surgery, such as valvular surgery, are unknown.

However, the leukocyte-specific effects shown in this trial may apply to other non-coronary cardiac surgical

scenarios.

Limitations

This study was not powered for clinical observations and conclusions cannot be drawn about

the relative effects of different methods of CPB on specific parameters of organ injury. The trial may also

have had insufficient power for the primary outcome. With hindsight, the decision to power the trial on cell

counts for the cantharidin blisters, rather than the primary outcome, may have been sub-optimal. The logic

for this decision was reasonable but the large target difference in cell counts (1.5 standard

deviations) may have been over-optimistic, even though based on pilot data). Using the relevant

correlations observed in the trial, we can now know that the trial was only able to detect a relatively large

standardised difference in ROS in granulocytes (about 0.85 standard deviations) with 90% power and a 2-

tailed significance level of 0.05.

Despite randomisation, diabetes was higher in cCPB which could, in principle, influence the parameters of

inflammation assayed in this study. The operating, anaesthetic and perfusion staff were not blinded to

allocations which, in theory, may have introduced unconscious performance biases.

However, aortic cross-clamp and total CPB exposure times for both cCPB and mCPB were similar in both randomised groups., which provides some evidence that the surgery proceeded in the same way apart from the method of CPB. Operativedifferences between groups were minimised by ensuring that all operations were carried out by a single surgeon. Aspirin may influence cell signalling in patients and anti-platelet use was an exclusion criteria. The pro-inflammatory signalling response of mCPB with concurrent aspirin treatment is unknown and is aconsideration for future studies.

CONCLUSION

Cardiac surgery with mCPB was associated with attenuation of pro-inflammatory intracellular signalling responses in leukocytes, measured by ROS and activation of p38-MAPK compared to cCPB. Accumulation of activated leukocytes into tissues and late markers of inflammation were equivalent between groups despite the alterations in signalling seen with mCPB.

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FIGURE LEGENDS

Figure 1: Summary of blood and blister fluid sampling times.

Blood and cantharidin-induced skin blister fluid were sampled in patients exposed to cCPB or mCPB. A time- line is presented to summarise sampling times.

Figure 2: Overview of participant selection.

Trial participants were recruited and assessed for exclusion criteria. Computer randomisation allocated participants to either mCPB (investigative group) or cCPB (control group).

Figure 3: Effect of CPB on ROS induction and phosphorylation of p38-MAPK and phosphorylation of NF-κB p65

CABG was performed with cCPB or mCPB. Peripheral blood samples were collected prior to surgery, post- induction (Post Ind), immediately prior to CPB (0 minutes) and at varying times following CPB initiation. Samples were stained using a ROS-sensitive dye (APF; A) antibodies that recognise phosphorylated p38- MAPK (B) or antibodies that recognise Ser529 phosphorylated NF-κB (C). Fluorescence of leukocyte cell subpopulations was quantified by flow cytometry after subtracting values from isotype-control antibodies. Data were pooled and log-transformed. Mean fluorescence intensity (MFI) values ± SD are shown with arbitrary units (AU).

Figure 4: Modelled data on log-transformed measurements of ROS, p38-MAPK and NF-κB p65

CABG was performed with cCPB or mCPB. Peripheral blood samples were collected prior to surgery, post- induction, immediately prior to CPB (0 minutes) and at varying times following CPB initiation. (A) ROS induction (B) phosphorylation of p38-MAPK or (C) phosphorylation at Ser529 of NF-κB p65 subunits was measured. Data were log-transformed the effects of CPB were estimated by

fitting mixed regression models. Mean fluorescence intensity (MFI) values ± SD are shown with arbitrary units (AU).

Figure 5: Extravasation of leukocytes into cantharidin skin blisters

CABG was performed with cCPB or mCPB. Blister fluid samples were collected prior to surgery (Baseline) and at 5 h following CPB initiation (post-operative). Cells were collected, stained using Diffquick and visualised by microscopy. Representative images are shown at ×40 optical magnification.

Figure 6: Summary of perioperative changes in CRP, White cell count, creatinine and haemoglobin clinical parameters

CABG was performed with cCPB or mCPB. Blood was sampled prior to surgery (baseline) and on the first and fourth days following surgery. Log-transformed data for (A) CRP, (B) white cell counts, (C) serum creatinine and (D) haemoglobin are presented ±SD.

Table 1. Patient characteristics

Values are expressed as mean with standard deviation or number with percentage in parentheses as indicated.

Variable	mCPB (n=13)	cCPB (n=13)
Female (n)	5 (38.4%)	3 (23.1%)
Age (years)	68.4 ± 6.8	66.3 ± 8.1
Body Mass Index (kg/m^2)	29.74 ± 4.53	29.31 ± 4.11
Logistic euroSCORE	3.35 ± 2.36	3.74 ± 3.14
Coronary vessel disease		
Single vessel disease	0	1 (8%)
Two vessel disease	1 (8%)	1 (8%)
Three vessel disease	12 (92%)	11 (84%)
Angina Status		
Asymptomatic	1 (8%)	1 (8%)
CCSI	0	0
CCS II	10 (77%)	7 (54%)
CCS III	1 (8%)	3 (23%)
CCS IV	1 (8%)	2 (15%)
Previous MI	3 (23%)	4 (31%)
Ejection fraction		
Good >50%	11 (85%)	12 (92%)
Fair 30-50%	2 (15%)	1 (8%)
Poor <30%	0	0
Diabetes		
Not diabetic	11 (85%)	6 (46%)
Diet controlled	0	0
Oral Therapy	1 (8%)	5 (38%)
Insulin	1 (8%)	2 (15%)
Smoking status		
Never smoked	6 (46%)	6 (46%)
Current smoker	1 (8%)	0
Ex-smoker	6 (46%)	7 (54%)
Hypertension		
Treated for BP>140/90	12 (92%)	12 (92%)
Chronic lung disease		
No	11 (85%)	12 (92%)
Yes	2 (15%)	1 (8%)
Extracardiac arteriopathy		
No	12 (92%)	12 (92%)
Yes	1 (8%)	1 (8%)
Pre-operative cardiac rhythm		
Sinus rhythm	11 (84%)	12 (92%)
Atrial fibrillation/flutter	1 (8%)	1 (8%)

Table 2. Peri- and post-operative data

Values are expressed as medians with inter-quartile range (IQR) or means with standard deviation or number with percentage in parentheses, as indicated.

Variable	mCPB (n=13)	cCPB (n=13)
Cardiopulmonary bypass time (min)	71 (IQR 62-84)	74 (IQR 67-91)
Total cross clamp time (min)	30 (IQR 24-33)	34 (IQR 29-38)
Number of coronary artery grafts	3.23 ± 0.62	3.23 ± 0.93
Transfusions in first 24 hours		
Packed red cells (units)	1.07 ± 1.38	1.15 ± 1.57
Platelets (pools)	0.15 ± 0.37	0.38 ± 0.51
Fresh frozen plasma (pools)	0.54 ± 1.05	0.46 ± 1.13
Time in ITU (days)	0.86 (IQR 0.80-1.33)	1.00 (IQR 0.80-1.10)
Hospital stay (days)	8 (IQR 5-11)	8 (IQR 7-13)
Post-operative neurological dysfunction		
No	13 (100%)	13 (100%)
Haemofiltration post-operatively		
No	13 (100%)	12 (92%)
Infective complications		
None	13 (100%)	10 (77%)
Harvest site infection	0	1 (8%)
Sternal wound infection	0	2 (15%)
Return to theatre		
No re-operation	13 (100%)	13 (100%)
Return for bleeding	0	0

Table 3. Treatment effects of mCPB vs cCPB for primary and secondary endpoints

[a]FITC Mean Fluorescent Intensity (MFI) / arbitrary units (AU); [b]PE-Cy7 MFI/AU; [c]PE MFI/AU; [d]cell counts per blister ($\times 10^5$). Other units otherwise indicated. *p-value for test of interaction of group and time.

		Difference between mCPB and cCPB (log units)	95% Confidence Interval	p value
Primary	ROS in granulocytes[a]	-0.053	-0.26 to 0.15	0.62
Secondary	ROS in monocytes[a]	0.010	-0.31 to 0.33	0.95
	ROS in lymphocytes[a]			
	0 mins	-0.092	-0.40 to 0.21	0.03*
	30 mins	-0.390	-0.69 to -0.09	0.55
	60 mins	-0.096	-0.40 to 0.21	0.01
	120 mins	0.049	-0.25 to 0.35	0.53
	300 mins	-0.006		0.75
			-0.31 to 0.30	0.97
	p38 in granulocytes[b]	-0.469	-0.89 to -0.05	0.03
	p38 in mononuclear cells[b]	0.493	-0.40 to 1.39	0.28
	p65 in granulocytes[c]	0.052	-0.24 to 0.35	0.73
	p65 in mononuclear cells[c]	0.171	-0.29 to 0.63	0.47
	Cantharidin blister polymorphonuclear cell count[d]	0.700	-0.34 to 1.74	0.17
	Cantharidin blister mononuclear cell count[d]	0.329	-0.39 to 1.05	0.34
	White cell count ($\times 10^9$/L)	0.064	-0.07 to 0.20	0.36
	Haemoglobin (g/dL)	-0.037	-0.10 to 0.02	0.23
	Creatinine (μmol/L)	-		0.04*
	Day 1 Creatinine (μmol/L)	0.050	-0.14 to 0.24	0.61
	Day 4 Creatinine (μmol/L)	-0.139	-0.33 to 0.05	0.16
	C-reactive protein (mg/mL)	0.135	-0.49 to 0.76	0.67

Table 4. White Blood Cell Count (WCC), Haemoglobin (Hb), Creatinine and C-Reactive Protein (CRP)

Values indicated are mean ±SD.

Time	WCC (×10⁹/L)		L)		Creatinin		CRP (mg/mL)		
	mCPB	**cCPB**	**mCPB**	**cCPB**	**mCPB**	**cCPB**	**mCPB**	**cCPB**	
Baseline	7.2 ± 1.4	7.8 ± 1.2	13.4 ± 1.1	13.2 ± 1.2	82.2 ± 1.3	93.8 ± 1.3	3 ± 2.67	2.27 ± 2.62	
Day 1	10.1 ± 1.2	9.2 ± 1.3	9.6 ± 1.1	10.0 ± 1.1	73.3 ± 1.3	80.0 ± 1.4	44.	08 ± 1.95	29.87 ± 6.05
Day 4	8.6 ± 1.3	8.9 ± 1.3	10.1 ± 1.1	10.3 ± 1.2	79.8 ± 1.3	103.3 ± 1.6	.08 ± 1.65	179.75 ± 1.53	

Table 5. Polymorphonuclear and mononuclear cantharidin blister cell counts

Values presented are means ± SD ($\times 10^5$ cells per blister).

Time	Polymorphonuclear cell count		count	
	mCPB	cCPB	mCPB	cCPB
Baseline	2.72 ± 3.08	3.15 ± 3.84	1.70 ± 1.54	2.06 ± 2.94
Post-operative	44.29 ± 2.63	22.58 ± 3.17	21.99 ± 1.51	15.73 ± 2.05

REFERENCES

1. Day, J.R. and K.M. Taylor, *The systemic inflammatory response syndrome and cardiopulmonary bypass.* Int J Surg, 2005. **3**(2): p. 129-40.

2. Remadi, J.P., et al., *Prospective randomized study comparing coronary artery bypass grafting with the new mini-extracorporeal circulation Jostra System or with a standard cardiopulmonary bypass.* Am Heart J, 2006. **151**(1): p. 198.

3. Perthel, M., et al., *Clinical advantages of using mini-bypass systems in terms of blood product use, postoperative bleeding and air entrainment: an in vivo clinical perspective.* Eur J Cardiothorac Surg, 2007. **31**(6): p. 1070-5; discussion 1075.

4. Immer, F.F., et al., *Minimal versus conventional cardiopulmonary bypass: assessment of intraoperative myocardial damage in coronary bypass surgery.* Eur J Cardiothorac Surg, 2005. **28**(5): p. 701-4.

5. Huybregts, R.A., et al., *Attenuated renal and intestinal injury after use of a mini-cardiopulmonary bypass system.* Ann Thorac Surg, 2007. **83**(5): p. 1760-6.

6. Ohata, T., et al., *Minimal cardiopulmonary bypass attenuates neutrophil activation and cytokine release in coronary artery bypass grafting.* J Artif Organs, 2007. **10**(2): p. 92-5.

7. Beghi, C., et al., *Mini-cardiopulmonary bypass system: results of a prospective randomized study.* Ann Thorac Surg, 2006. **81**(4): p. 1396-400.

8. Immer, F.F., et al., *Minimal extracorporeal circulation is a promising technique for coronary artery bypass grafting.* Ann Thorac Surg, 2007. **84**(5): p. 1515-20; discussion 1521.

9. Evans, B.J., et al., *The inflammatory effect of cardiopulmonary bypass on leukocyte extravasation in vivo.* J Thorac Cardiovasc Surg, 2008. **135**(5): p. 999-1006.

10. Momin, A., et al., *Miniaturized cardiopulmonary bypass: the Hammersmith technique.* J Cardiothorac Surg, 2013. **8**: p. 143.

11. Nguyen, B.A., et al., *Metabolic derangement and cardiac injury early after reperfusion following intermittent cross-clamp fibrillation in patients undergoing coronary artery bypass*

graft surgery using conventional or miniaturized cardiopulmonary bypass. *Mol Cell Biochem*, 2014. **395**(1-2): p. 167-75.

12. Bubici, C., et al., *Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: molecular basis and biological significance.* Oncogene, 2006. **25**(51): p. 6731-48.

13. Raingeaud, J., et al., *MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway.* Mol Cell Biol, 1996. **16**(3): p. 1247-55.

14. Baltimore, D., *NF-kappaB is 25.* Nat Immunol, 2011. **12**(8): p. 683-5.

15. Kiaii, B., et al., *The early inflammatory response in a mini-cardiopulmonary bypass system: a prospective randomized study.* Innovations (Phila), 2012. **7**(1): p. 23-32.

16. Fromes, Y., et al., *Reduction of the inflammatory response following coronary bypass grafting with total minimal extracorporeal circulation.* Eur J Cardiothorac Surg, 2002. **22**(4): p. 527-33.

17. Jacobs, S., et al., *Limitations for manual and telemanipulator-assisted motion tracking--implications for endoscopic beating-heart surgery.* Ann Thorac Surg, 2003. **76**(6): p. 2029-35; discussion 2035-6.

18. van Boven, W.J., et al., *Mini extracorporeal circuit for coronary artery bypass grafting: initial clinical and biochemical results: a comparison with conventional and off-pump coronary artery bypass grafts concerning global oxidative stress and alveolar function.* Perfusion, 2004. **19**(4): p. 239-46.

19. Scarci, M., et al., *Does intermittent cross-clamp fibrillation provide equivalent myocardial protection compared to cardioplegia in patients undergoing bypass graft revascularisation?* Interact Cardiovasc Thorac Surg, 2009. **9**(5): p. 872-8.