Effects of propofol and isoflurane on haemodynamics and the inflammatory response in cardiopulmonary bypass surgery

S. SAYED*, N. K. IDRISS†, H. G. SAYYED‡, A. A. ASHRY§, D. M. RAFATT#, A. O. MOHAMED† and A.D. BLANN¥

*Departments of *Anaesthesiology, †Medical Biochemistry, ‡Medical Physiology, §Cardiothoracic Surgery and #Pediatrics. Faculty of Medicine, Assiut University, Assiut, Egypt; and ¥Department of Medicine, University of Birmingham Centre for Cardiovascular Science, City Hospital, Birmingham B18 7QH, UK*

Accepted: 20 May 2015

ABSTRACT

Cardiopulmonary bypass (CPB) causes reperfusion injury that when most severe is clinically manifested as a systemic inflammatory response syndrome. The anaesthetic propofol may have anti-inflammatory properties that may reduce such a response. We hypothesised differing effects of propofol and isoflurane on inflammatory markers in patients having CBP. Forty patients undergoing elective CPB were randomised to receive either propofol or isoflurane for maintenance of anaesthesia. CRP, IL-6, IL-8, HIF-1α (ELISA), CD11 and CD18 expression (flow cytometry), and haemoxygenase (*HO-1*) promoter polymorphisms (PCR/electrophoresis) were measured before anaesthetic induction, 4 hours post-CPB, and 24 hours later. There were no differences in the 4 hours changes in CRP, IL-6, IL-8 or CD18 between the two groups, but those in the propofol group had higher HIF-1α ($P=0.016$) and lower CD11 expression ($P=0.026$). After 24 hours, compared to the isoflurane group, the propofol group had significantly lower levels of CRP ($P<0.001$), IL-6 ($P<0.001$) and IL-8 ($P<0.001$), with higher levels CD11 ($P=0.009$) and CD18 ($P=0.002$) expression. After 24 hours, patients on propofol had increased expression of shorter *HO-1* GT(n) repeats than patients on isoflurane ($P=0.001$). Use of propofol in CPB is associated with a less adverse inflammatory profile than is isofluorane, and an increased up-regulation of *HO-1*. This supports the hypothesis that propofol has anti-inflammatory activity.

KEY WORDS: Cardiopulmonary bypass.
Hemoxygenase.
Inflammation.
Isoflurane.
Propofol.

Introduction

Cardiopulmonary bypass (CPB) is coupled with a complex physiological response that is clinically manifested as a non-specific generalised inflammatory state described as systemic inflammatory response syndrome.[1,2] Clinical consequences vary from mild (with limited effects on the patient's condition such as an acute phase response) to severe (with multiple organ failure).[3] The inflammatory response, as measured by increased plasma CRP, is activated by a number of mechanisms such as contact of blood with the synthetic circuit, hypoxia, and ischaemia reperfusion following release of the aortic cross clamping, and is linked with increased levels of circulating cytokines and with leucocyte activation.[4,5]

Several drugs used in the induction and maintenance of anaesthesia, and the maintenance of postoperative sedation and analgesia are thought to possess immunomodulatory effect.[6] Commonly used agents include propofol (2,6-diisopropylphenol) and isoflurane. Clinical and animal models indicate that these have anti-inflammatory properties, such as decreasing pro-inflammatory cytokine production (such as IL-6 and IL-8) and inhibiting neutrophil function.[7–10] In addition, these molecules have been reputed to play a role in neutrophil activation, degranulation and up-regulation of adhesion molecules (CD11/CD18) during thoracic surgery.[11–13] Plasma levels of hypoxia-inducible factor-1α (HIF-1α) rise in response to ischaemia, and have roles in cell metabolism, angiogenesis, and cardiac function.[14,15]

Haemoxygenase (HO-1), the rate limiting enzyme in the degradation of haem to bilirubin, iron and carbon monoxide, is believed to have a beneficial effect on the cardiovascular system. The 5′-flanking region of the HO-1 gene (*HO-1*) has a variable number of GT repeats 526 bp upstream of the transcription site.[16,17] The number of GT repeats (GT[n]) controls the inducibility of the promoter under oxidative stimuli; the shorter polymorphic allele with a small number of GT(n) repeats (typically $n<25$) leads to high *HO-1* inducibility, whereas a high number of GT(n) repeats is linked to reduced inducibility.[18] Thus the number of GT(n) repeats, and so the overall length of the overall GT sequence, exerts potentially protective effects against the effects of oxidative stress. This may have clinical relevance, since type 2 diabetics carrying a high number of GT(n) repeats could be risk of higher oxidative stress that may in turn (it is speculated) lead to an increased susceptibility to the development of cardiovascular disease,[19] while those

Correspondence to: Dr. Andrew D. Blann
Blood Science Solutions, Crabtree Road, Birmingham B18 7JT, UK
Email: andrew.blann@hotmail.com

patients with advanced peripheral atherosclerosis carrying a short GT(n) allele are protected from major cardiovascular events such as myocardial infarction.[20] Furthermore, induction of HO-1 by isoflurane protects rat livers from ischaemic/reperfusion injury.[21]

Our objective was to test the hypothesis that propofol has a more beneficial action on inflammatory responses in CPB than does isoflurane. We defined beneficial action by changes in plasma levels of CRP, IL-6, IL-8, HIF-1α, and the expression of CD11 and CD18 by leucocytes.

Material and methods

Subjects

Inclusion criteria were adult patients about to undergo elective CPB for valve repair or coronary artery bypass grafting in Assiut University Hospital who were able to give written informed consent. Exclusion criteria were previous cardiac surgery, ejection fraction <40%, requirement for inotropic drugs preoperatively, chronic renal insufficiency (creatinine >1.6 mg/dL) or chronic renal failure requiring dialysis, preoperative use of steroids, age <18 years, or weight 50% above or below ideal body weight. The study was approved by the local ethics committee, and written informed consent was obtained from each patient. Under these conditions we recruited and entered 46 patients into the study. Routine preoperative and postoperative measures included full blood cell, coagulation profile, liver function tests, fasting blood sugar, creatinine, urea and electrolytes (sodium, potassium, calcium, magnesium), temperature and haemodynamic data (heart rate, mean arterial blood pressure, and central venous pressure). Some of these were collected serially in the immediately after surgery. Body mass index was calculated as weight (in kilograms) divided by height (in metres) squared.

Anaesthetic technique

All patients were premedicated with IV midazolam (0.03 mg/kg) 30 min before the operation. After establishing standard monitoring procedures, anaesthesia was induced with fentanyl (3 μg/kg) combined with propofol (1–2 mg/kg). Endotracheal intubation was performed after achieving muscle relaxation with rocuronium bromide (1.0 mg/kg). Mechanical ventilation was instituted to maintain eucapnia. In the propofol group (n=20), anaesthesia was maintained using continuous infusion of fentanyl (1 μg/kg/h) and propofol (3–8 mg/kg/h). In the isoflurane group (n=20), anaesthesia was maintained using continuous infusion of fentanyl (1 μg/kg/h) and isoflurane (2%) according to clinical requirement. During CPB, isoflurane inhalation was discontinued and anaesthesia maintained using a propofol infusion. The A-line ARX index (AAI) was used by AEP-monitor/2 to monitor the depth of anaesthesia so that the propofol infusion was titrated or additional isoflurane given. Fentanyl infusion of 1 μg/kg/h was continued post-operatively in the intensive care unit.

Surgery

All patients had a median sternotomy. An initial dose of 400 units/kg heparin was used to obtain an activated clotting time (ACT) >450 sec before CPB, which was instituted with a non-pulsatile heart-lung machine. Mean blood pressure of 50–70 mm Hg and blood flow of 2.0–2.4 L/min/m^{-2} were maintained during CPB. The priming solution contained mannitol and heparin. Moderate hypothermic CPB (28–32°C) was used in all patients. Cardiac arrest was achieved and maintained using a cold cardioplegic solution at 4°C. Theatre and ICU staff were aware of the randomisation group of their patient.

Haemodynamic data (heart rate, mean arterial blood pressure, and central venous pressure), were collected: immediately pre-induction (Tbaseline); 30 min post-induction (T1); 30 min post-bypass (T2); 1 h post-bypass (T3); 2 h post-bypass (T4); upon ICU admission (T5); and 3 h (T6) and 6 h (T7) after ICU admission. After surgery, all patients were admitted to ICU where data were collected by staff unaware of patient group. Body temperature was measured on arrival to the ICU and every 2 h for the next 10 h. Before being discharged, patients had to meet the following three criteria: 1) aware; 2) impulsive breathing and detached endotracheal tube; 3) stable haemodynamics without vasoactive drugs. Tracheal intubation time, and length of ICU and hospital stay were recorded. Standardised post-operative drugs were paracetamol (acetaminophen) (infusion IV 1 g every 8 h) and the antibiotics were

Table 1. Demographic data and peri-operative characteristics of cardiopulmonary bypass patients.

	Propofol group	Isoflurane group	*P*
Age (years)	43 (12)	47 (9)	0.243
Males/female (*n*)	16/4	18/2	0.376
Body mass index (kg/m^2)	25.6 (5.8)	26.4 (4.9)	0.643
Type of surgery: Valve replacement/Coronary artery bypass grafting	7/13	13/7	0.058
Bypass time (min)	149 (4)	154 (10)	0.055
Cross-clamping time (min)	121 (2)	121 (5)	0.768
Surgery duration (min)	382 (24)	362 (37)	0.051
Duration of tracheal intubation (h)	9.4 (1.2)	9.7 (1.4)	0.561
Length of ICU stay (days)	2.5 (0.8)	3.1 (0.8)	0.013
Length of post-operative ward stay (days)	11.2 (1.8)	11.7 (1.0)	0.295

Data expressed as mean (standard deviation [SD]) or number (*n*) and analysed by Student's *t*-test or χ^2 test.
ICU: intensive care unit.

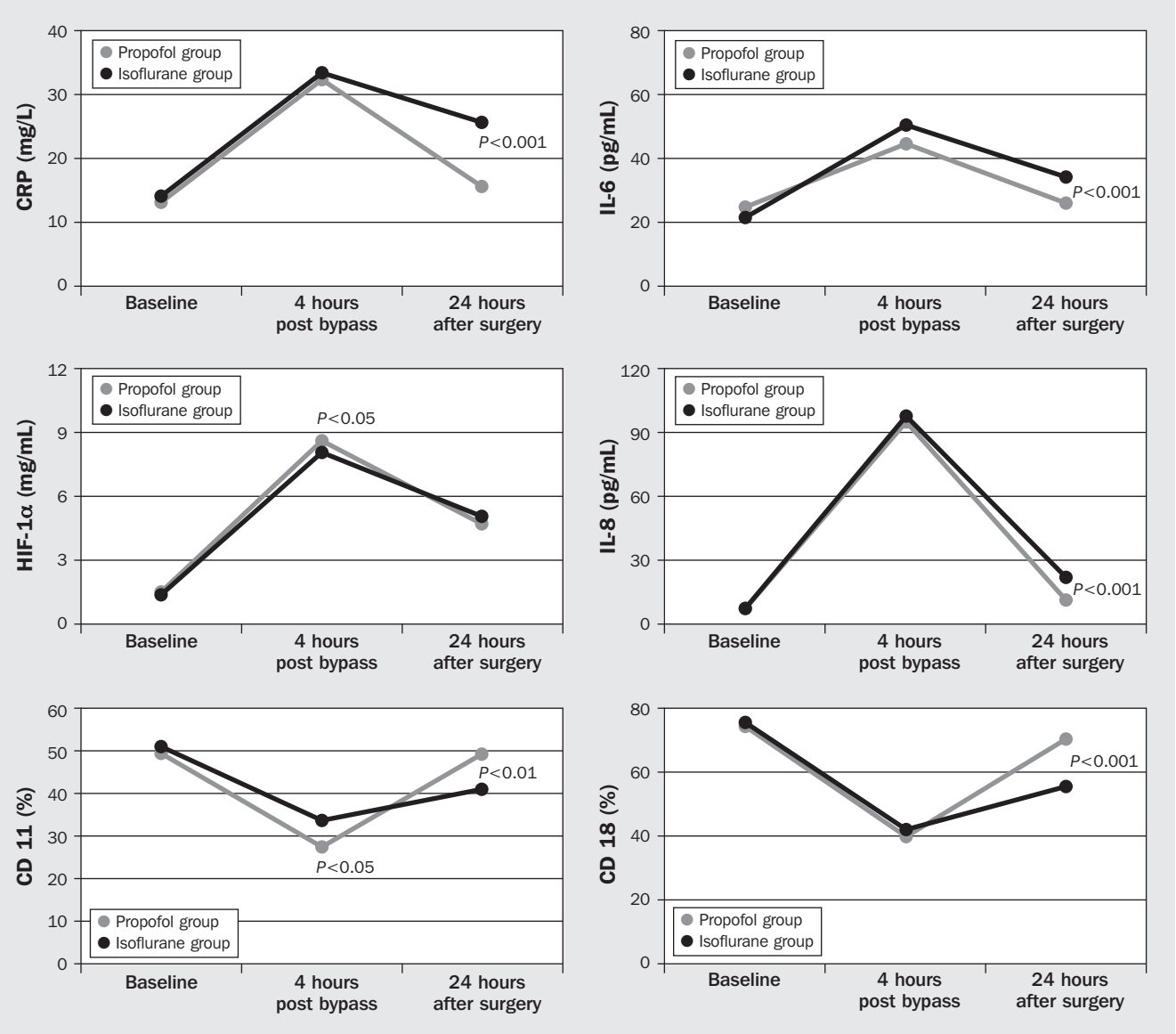

Fig. 1. Changes in inflammatory mediators and expression of adhesion molecules. Black line: patients on propofol. Grey line: patients on isoflurane. CRP: C-reactive protein; IL-6: interleukin-6, IL-8: interleukin-8, HIF-1α: hypoxia-inducible factor-1 . *P* values refer to difference between the propofol and isoflurane groups at the particular time point.

ceftazidine (Fortum) (1 g /12h IV). Steroids were not used. Patients were warmed using forced air warming blankets and IV 0.9% saline warmed to body temperature (Hotline Blood and Fluid Warmer, Smiths Medical, London UK).

Laboratory

For the six research indices, from each participant 10 mL blood was drawn from the central venous catheter (CVC), immediately before induction of anaesthesia (baseline), 4 h post-CPB, and after 24 h. Venous blood samples were collected in tubes containing Na_2EDTA. Care was taken to prevent any mechanical damage which might cause haemolysis of the blood. Some tubes were allowed to stand at room temperature for few minutes then centrifuged at 3000 x*g* for 15 min. The clear supernatants were quickly removed and stored at −70°C for enzyme-linked immunosorbent assays (ELISA) measurement of CRP (Immunodiagnostic AG, USA), IL-6 (Assaypro LLC, EI1006-1, www.assaypro.com), IL-8 (Glory Science, CK-E10139) and HIF-1α (Glory Science, CK-E10306), and following the instructions supplied with each kit.

For flow cytometric analysis of CD11 and CD18, blood was drawn before the start of CPB (baseline), at 4 h after post-CPB and 24 h after surgery. Tubes were placed on ice and analysed within 2 h of venepuncture. Ninety μL whole blood and 10 μL primary antibody (100 μg/mL) were mixed in 12×75 mm polystyrene tubes (Falcon, Becton Dickinson UK, Cowley, UK) for 15–30 min. After labelling unbound antibody was removed by centrifugation, and each antibody was controlled at each time point by an extraneous isotypic control. After two washes with phosphate-buffered saline, fluorescein isothiocyanate-conjugated secondary antibodies were added at the manufacturer's recommended concentration (Sigma Chemical, Dorset, UK) and incubation was sustained for a further 15 min. Red blood cells were lysed for 1 min by the addition of 1 mL Coulter whole blood lysing reagent and fixed in 250 μL fixative solutions (Coulter Electronics, Luton, UK). Lysed samples were sealed in 5%

formaldehyde and interpret on a flow cytometer (EPICS XL, Coulter Electronics). Neutrophils were identified by their characteristic forward and side scatter profiles. Amplifier gains were regulated with beads before each experiment. Five-thousand events were acquired for each sample. The neutrophils were analysed for strength of fluorescence by mathematically adapting the logarithmic mean fluorescence values attained from the histograms into relative mean fluorescence values. The values reported are as a percentage change from the observed baseline values. Laboratory work was performed without knowledge of the patient's randomisation group.

Molecular genetics

Genomic DNA was extracted from venous blood with the use of a QIA amp blood kit (Qiagen) according to the manufacturer's protocol. Puregene (DNA isolation kit, Gentra System, Minneapolis, MN, USA)] Briefly, DNA was isolated and loaded onto a QIAamp spin column, washed twice and extracted in 200 μL distilled water. Genomic DNA was purified from human whole blood samples with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The 5'-flanking region containing the GT(n) repeats of *HO-1* was amplified by the polymerase chain reaction (PCR) with a FAM-labelled sense primer (5`-AGAGCCTGCAGCTTCTCAGA-3 ) and an unlabeled antisense primer (5`-ACAAAGTCTGGCCATAGGAC-3). The PCR cycle of 94˚C for 30 sec, 57°C for 30 sec and 72˚C for 30 sec was carried out for a total of 30 cycles. The PCR harvest was then run on a denaturing polyacrylamide gel (6%, acrylamide: bis-acrylamide 19:1) at 2000 V for 2 h, followed by autoradiography.

The sizes of the PCR products were analysed with ABI prism 310 by using software for DNA sequence assembly/alignment, sequenced by the dideoxy chain termination method of Sanger *et al*. Initially, a sequencing reaction purification used a Centri–Sep column kit protocol, briefly as follows. A dry gel was rehydrated with 800 μL and left 30 min to set and 20 μL dye terminator reaction was transferred to the top of the gel. 15 μL Hi-Di formamide was added to the purified sample, mixed, and heated at 95˚C for 2 min, chilled on ice, then loaded onto the 310 ABI sequencer instrument. Single-stranded DNA templates were recovered. Compilation of sequence data and DNA sequence analysis were accomplished by using PC Gene software, version 6.01 (Intelligenetics, Mountain View, California.). The respective sizes of the GT(n) repeats for each participant were then calculated using Gene Mapper version 3.0 (ABI Prism). The number of GT(n) repeats was determined in the sample before anaesthesia, and in the sample 24 h after the completion of the surgery. Laboratory work was performed without knowledge of the patient's randomisation group.

Progress of the study

Six patients were excluded from the study after randomisation. These were two cases in the isoflurane group who suffered from inadequate sedation post-operatively and required addition of propofol infusion. The remaining four patients were in the propofol group: these were two

Table 2. Comparison of the effect of propofol and isoflurane on laboratory parameters in cardiopulmonary bypass patients.

Parameters	Propofol group		Effect of surgery	Isoflurane group		Effect of surgery	Between groups effect
	Before surgery	After surgery	*P*	Before surgery	After surgery	*P*	*P*
WBCC ($x10^6$/mL)	8.0 (2.1)	8.5 (2.6)	0.626	8.8 (2.2)	7.7 (2.1)	0.226	0.156
RBCC ($x10^9$/mL)	4.7 (0.6)	3.5 (0.4)	<0.001	4.6 (0.6)	3.6 (0.7)	0.001	0.241
Platelets ($x10^6$/mL)	285 (72)	206 (45)	<0.001	237 (52)	185 (25)	0.001	0.130
Prothrombin time (s)	13.6 (2.2)	20.6 (5.9)	<0.001	14.8 (2.3)	18.5 (6.1)	0.024	0.159
Creatinine (μmol/L)	76 (10)	111 (14)	<0.001	74 (8)	107 (23)	<0.001	0.498
Urea (mmol/L)	5.9 (0.6)	3.1 (1.4)	<0.001	5.7 (1.3)	3.5 (1.7)	<0.001	0.620
Sodium (mmol/L)	147 (11)	135 (10)	<0.001	149 (9)	133 (9)	<0.001	0.840
Potassium (mmol/L)	5.0 (0.4)	3.4 (0.5)	<0.001	5.0 (0.4)	3.5 (0.7)	<0.001	0.383
AST (U/L)	35 (4)	48 (9)	0.001	35 (3)	44 (7)	<0.001	0.242
ALT(U/L)	34 (13)	53 (13)	<0.001	33 (12)	48 (15)	<0.001	0.367
ALP(U/L)	0.66 (0.19)	0.95 (0.25)	0.002	0.73 (0.23)	0.88 (0.26)	0.03	0.182
Albumin (g/L)	34 (12)	24 (8)	<0.001	35 (11)	27 (10)	<0.001	0.471
Total protein(g/L)	75 (10)	66 (7)	<0.001	62 (10)	62 (10)	<0.001	0.099
Direct bilirubin (μmol/L)	3.6 (0.9)	6.5 (1.5)	<0.001	3.8 (0.9)	6.6 (1.2)	<0.001	0.841
Magnesium (mmol/L)	1.3 (0.3)	1.6 (0.4)	0.022	1.2 (0.3)	1.4 (0.3)	0.021	0.742
Free calcium (mmol/L)	1.2 (0.1)	1.3 (0.2)	0.131	1.2 (0.2)	1.2 (0.1)	0.793	0.135
Glucose (mmol/L)	5.6 (0.9)	11.8 (1.9)	<0.001	5.3 (0.9)	11.1 (2.2)	<0.001	0.503

RBCC: red blood cell count; WBCC: white blood cell count; AST: aspartate transaminase;
ALT: alanine transaminase; ALP: alkaline phosphatase.
Data expressed as mean (SD) and analysed by paired *t*-test.

Table 3. Changes in haemodynamics.

	Time point	Propofol	Isoflurane	*P*
Heart rate(bpm)	Pre-induction	89 (11)	87 (8)	0.515
	30 min post-induction	84 (7)	79 (7)†	0.021
	30 min post-CPB	79 (7)*	75 (8)*	0.201
	60 min post-CBP	94 (10)	102 (7)*	0.010
	2 h post-CPB	81 (8)†	84 (9)	0.293
	Upon ICU entry	83 (7)‡	81 (8)	0.510
	3 hours after entry	85 (8)	81 (8)	0.113
	6 hours after entry	86 (6)	84 (7)	0.322
Mean arterial pressure(mm Hg)	Pre-induction	86 (8)	89 (5)	0.099
	30 min post-induction	78 (8*	82 (4)*	0.042
	30 min post-CPB	72 (6)*	73 (6)*	0.490
	60 min post-CBP	67 (6)*	77 (5)*	<0.001
	2 hours post-CPB	70 (5)*	82 (3)*	<0.001
	Upon ICU entry	73 (4)*	84 (3)†	<0.001
	3 hours after entry	76 (4)*	85 (5)†	<0.001
	6 hours after entry	70 (5)*	82 (3)*	<0.001
Central venous pressure(mm Hg)	Pre-induction	9.3 (0.4)	9.2 (0.4)	0.501
	30 min post-induction	9.3 (0.7)	9.0 (0.4)	0.136
	30 min post-CPB	9.1 (0.4)	8.9 (0.4)	0.242
	60 min post-CBP	8.0 (0.5)*	8.0 (0.4)*	0.922
	2 hours post-CPB	8.8 (0.5)‡	8.5 (0.4)*	0.108
	Upon ICU entry	8.9 (0.7)	8.8 (0.4)†	0.514
	3 hours after entry	8.8 (0.6)‡	8.9 (0.6)‡	0.761
	6 hours after entry	8.8 (0.4)‡	8.8 (0.5)†	0.882

Serial data (mean [SD]) analysed by repeated measures ANOVA/general linear model with the pre-induction data as reference: *$P<0.001$, †$P<0.01$, ‡$P<0.05$.
bpm: beats per minute. *P* value between groups by *t*-test.

cases that suffered from post-operative bleeding and required re-exploration, one case transferred from the operation room to the ICU with intra-aortic balloon and who died post-operatively, and one case who suffered from repeated episode of supraventricular tachycardia and required further midazolam sedation. The study was continued until there were 20 completed patients in each group. No patient withdrew their consent.

Statistics and analysis

We hypothesised a difference in the pattern of response of any of the six primary research indices (CRP, IL-6, IL-8, HIF-1α, CD11 or CD18) of 20% between time points. Accordingly, we modelled a mean 100 units/mL (standard deviation [SD]) 30 units/mL at one time point (baseline), changing to 80 (30) units/mL at a second, and changing further to 60 (30) units/mL at a third. To achieve this at $P<0.001$ overall (repeated measures analysis of variance) and $P<0.01$ between the first and third time point (Tukey's post-doc test), a sample size of $n=20$ is required. This total sample size of 40 exceeds those of others reporting changes in plasma and cell indices (such as inflammatory cytokines) before and after surgery.[10–12] The sample size also brings the power to robustly defend a correlation coefficient of >0.6 at $2P<0.05$ and 1-beta = 0.8.

Data with a continuous variation are expressed as mean (SD) and compared using paired or unpaired *t*-tests if normally distributed, or as median (inter-quartile range) and compared by Mann-Whitney U test or Wilcoxon's test if non-normally distributed. Categorical data was compared by the χ^2 test. Data at multiple time points was analysed by two-way (repeated measured) analysis of variance with Tukey's post-hoc test in a general linear model. Analyses were performed on Minitab 16 and $P<0.05$ was considered significant.

Results

Clinical, demographic and surgical data are presented in Table 1, routine laboratory indices in Table 2. As expected, there were multiple differences in most indices before and after surgery, as per normal surgical practice (e.g., anticoagulation, glucose infusion) but there were no inter-group differences, although patients on propofol spent 0.6 fewer days in ICU than patients on isoflurane.

Table 3 shows haemodynamic data. In the propofol group, the heart rate fell from a mean of 89 beats per min pre-induction to a rate of 79 at 30 min after CBP, to a rate of 81 at 2 h after CBP, and upon entry to the ICU, when the rate was 83 beats per min. In the isoflurane group, heart rate was 87 beats per min pre-induction, but this rate fell to 79 and then

to 75 beats per min at 30 and 60 min post-CBP, respectively. The heart rate then increased to 102 beats per min 60 min after CBP. Mean arterial pressure was lower than at pre-induction at all time points in both groups, but the difference was significant 30 min post-induction, and at 60 min post-induction and beyond. Compared to pre-induction, central venous pressure was lower 60 min post-CPB, 2 h post-CPB and at 3 and 6 h after ICU entry in the propofol group. In the isoflurane group, central venous pressure was lower 60 min after CBP and thereafter. There were no differences between the two groups of patients.

Table 4 shows changes in temperature, which were consistently higher at all ICU times in the isoflurane group (median increase 0.3°C, IQR 0.1–0.6, $P<0.001$). In the propofol group, there was no significant difference in the temperature between pre-anaesthesia and arrive in ICU. In contrast, in the isoflurane group, temperature was higher upon arrival at ICU compared to pre-anaesthesia. The median (IQR) length of ICU stay was 2.0 (2.0–3.0) days in the propofol group and 3.0 (2.25-4.0) days in the isoflurane group ($P=0.0248$, Mann-Whitney U test). Similarly, the length of post-operative ward stay was 11 (10–12) days in the propofol group and 12 (11–12.75) days in the isoflurane group ($P=0.1404$). Factors such as wound infection, use of antibiotics and differences in use of vasopressors could influence these data.

Table 5 shows levels of inflammatory markers, expression of leucocyte adhesion molecules and HIF-1 . CRP was raised in the 4 h and 24 h samples in both groups of patients, but was higher at 24 h in the isofluane group. IL-6 and IL-8 levels were both raised in the propofol group at 4 h but at 24 h the levels had returned to baseline. However, in the isoflurane group, levels of both cytokines were still raised after 24 h. Leucocyte expression of CD11 and CD18 both fell after 4 h in the propofol group, but were restored in the 24 h samples. In the isoflurane group, expression of the markers also fell at 4 h but were still low at 24 h. Levels of HIF-1α were increased at 4 h and 24 h in both groups. However, levels were higher in the propofol group at 4 h than in the isoflurane group (Fig. 2). The mean (SD) number of *HO-1* GT(n) repeats in the propofol group at baseline was 27.8 (3.2), falling to 22.2 (2.4) 24 h after the completion of surgery ($P<0.001$). In the isoflurane group, the number of repeats was 28.3 (3.0), which fell to 25.6 (3.6) ($P=0.048$). The number of repeats after the procedure was lower in the propofol group than in the isoflurane group.

The change in the number of GT(n) repeats in the propofol group correlated inversely with the change in IL-6 levels after 24 h ($r= -0.59$, $P=0.006$). The change in the number of GT(n) repeats in the isoflurane group also correlated inversely with the change in IL-6 levels after 24 h ($r= -0.67$, $P=0.001$). There were no significant relationships between change in GT(n) and IL-8 in either group (propofol $r=0.21$, $P=0.369$; isoflurane $r= -0.36$, $P=0.117$).

Table 4. Changes in temperature.

Time point	Propofol group	Isoflurane group	Difference between groups (*P*)
Pre-anaesthesia	36.6 (0.5)	36.5 (0.3)	0.439
Arrival in ICU	36.8 (0.5)*	37.3 (0.2)†	<0.001
+2 h	37.1 (0.3)†	37.3 (0.3)†	0.012
+4 h	37.2 (0.2)†	37.5 (0.2)†	<0.001
+6 h	37.2 (0.3)†	37.5 (0.2)†	<0.001
+8 h	37.3 (0.2)†	37.5 (*0.2)†	<0.001
+10 h	37.2 (0.2)†	37.6 (0.1)†	<0.001

ICU: intensive care unit.

Data expressed as mean (SD) analysed by repeated measures ANOVA/general linear model with the pre-anaesthesia data as reference (*$P=0.125$, †$P<0.001$) compared to pre-anaesthesia.

P value between groups by *t*-test.

Discussion

A major consequence of cardiopulmonary bypass is a systemic inflammatory response that may induce tissue damage and multiple organ dysfunction.[1–3] Thus, use of agents that inhibit the release of inflammatory cytokines is likely to be an important therapeutic tool.[4–9] We hypothesised a difference in the pattern of the inflammatory response to surgery in those treated with propofol versus those treated with isoflurane. Use of propofol was linked to lower CRP, IL-6, IL-8, and a more rapid normalisation of adhesion molecule expression, and to higher HIF-1α four hours after induction. Propofol was also associated with a greater change in the number of *HO-1* GT(n) repeats than isoflurane, but both changes correlated inversely with levels of IL-6 (but not IL-8) at 24 h.

Numerous studies have compared propofol with isoflurane and other anaesthetics,[22–25] and it has been suggested that anaesthesia may also influence the systemic inflammatory response by suppressing the release of cytokine mediators.[5–9] In support of this hypothesis are reports that propofol is linked reduced IL-6, IL-8 and CRP compared to fluranes in a variety of major surgery settings.[26–29] We concur with data of Liu[26] in that we too found that propofol is better at inhibiting IL-8 than isoflurane, and extend this observation to IL-6 and CRP. Yoo *et al*.[27] reported that propofol produced lower levels of IL-6 and CRP than sevoflurane, as did we with isoflurane, while Ke *et al*.[28] found that propofol plus remifentanil was linked to lower IL-6 levels than isoflurane. Corcoran *et al*.[29] compared propofol with saline: the former was linked to reduced IL-6 and IL-8 after CABG/CPB unclamping, and the authors speculated that this effect was due to reduced oxidant activity as defined by serum malondialdehyde. In contrast, Baki *et al*. found that propofol was associated with higher levels of IL-6 and IL-8 after CPB for CABG than was desflurane, although there was no difference in changes in TNFα.[30]

Adhesion molecules expressed on cell surface of leucocytes play a vital role in the recruitment of these cells to the site of inflammation by mediating leucocyte rolling, adhesion, and intraluminal migration. Various groups have used neutrophil CD11 and CD18 expression as surrogates of their participation in inflammatory responses. Our finding of reduced CD11 and CD18 after CPB reflects that of Murphy *et al*.,[11] while cardiovascular surgery in children induced more evident and continued reduced expression of CD11a/CD18 and CD11b/CD18[31] and CD11c.[32]

Table 5. Comparison of the effect of propofol and isoflurane on inflammatory markers (CRP, L-6 and IL-8), adhesion molecules (CD11 and CD18) and HIF-1α.

	Propofol group		Isoflurane group		
Analyte	Result	*P* from baseline	Result	*P* from baseline	Between groups *P*
CRP (mg/L)					
Baseline	13.1 (1.9)	Reference	14.0 (1.8)	Reference	0.125
4 h	32.3 (3.0)	<0.001	33.6 (2.4)	<0.001	0.146
24 h	15.7 (4.0)	0.021	25.8 (3.2)	<0.001	<0.001
IL-6 (pg/mL)					
Baseline	24.5 (6.4)	Reference	21.8 (4.4)	Reference	0.124
4 h	45.4 (10.3)	<0.001	51.2 (8.6)	<0.001	0.064
24 h	25.8 (4.4)	0.849	34.5 (6.1)	<0.001	<0.001
IL-8 (pg/mL)					
Baseline	7.1 (0.9)	Reference	6.9 (1.8)	Reference	0.764
4 h	94.0 (14.2)	<0.001	98.1 (14.5)	<0.001	0.371
24 h	11.8 (1.4)	0.176	21.7 (3.4)	<0.001	<0.001
HIF-1α (ng/mL)					
Baseline	1.42 (0.37)	Reference	1.37 (0.36)	Reference	0.197
4 h	8.74 (0.98)	<0.001	8.07 (0.65)	<0.001	0.016
24 h	4.72 (0.88)	<0.001	5.12 (1.0)	<0.001	0.190
CD11 (%)					
Baseline	49.8 (10.3)	Reference	50.9 (10.9)	Reference	0.753
4 h	27.5 (7.0)	<0.001	33.7 (9.7)	<0.001	0.026
24 h	49.1 (10.0)	0.967	40.8 (8.9)	0.006	0.009
CD18 (%)					
Baseline	74.5 (11.3)	Reference	76.0 (16.0)	Reference	0.731
4 h	39.3 (7.1)	<0.001	41.5 (5.9)	<0.001	0.294
24 h	70.0 (16.7)	0.386	55.4 (8.6)	<0.001	0.002

In all cases overall repeated measures analysis of variance was $P<0.001$. Data expressed as mean (SD). CRP: C-reactive protein; IL-6: interleukin-6; IL-8: interleukin-8; CD11: adhesion molecule CD11; CD18: adhesion molecule CD18; HIF-1α: hypoxia-inducible factor 1α. *P* from baseline by a general linear model with Tukey's *post-hoc* test. *P* between groups by paired *t*-test. Baseline sample taken pre-anaesthesia; 4-h sample taken at completion of surgery; 24-h sample taken 24 h after the end of surgery while in ICU.

Unfortunately, we cannot compare our data to that of Corcoran *et al*.[29] as their comparator to propofol was saline and we looked at different time windows. Our contribution is to note that the expression of both adhesion molecules returned to normal at 24 h in those on propofol, whereas expression was still lower than at baseline in those on isoflurane.

Hypoxia-inducible factor-1α (HIF-1α) controls the expression of several genes concerned with homeostatic responses to hypoxia, and raised HIF-α is a risk factor for adverse cardiac outcome.[14,15] Tanaka *et al*.[33] found that propofol inhibited HIF-1α activation induced by lipopolysaccharide in macrophages *in vitro*, and speculated that propofol inhibits cellular inflammatory reaction through suppression of this cytokine. Moreover, Yeh *et al*.[34] showed, in an animal model, that propofol protected the lung against sepsis through inhibition of HIF-1α. We extend these data, showing that propofol and isoflurane suppress the increase of HIF-1α at 24 h post-CPB after the initial increase at 4 h but these changes were not significant.

Increased activity of *HO-1* and plasma levels of HO-1 are believed to have a role in cardiovascular disease and inflammation,[16–21,35–37] Propofol promoted high HO-1 expression by cardiac tissue in children with congenital heart disease undergoing cardiac surgery, and propofol increases *HO-1* expression *in vitro*.[38,39] In an animal model, propofol increased cardiomyocyte HO-1, which may have been linked to protection from hyperglycaemia-mediated hypertrophy and apoptosis.[40] Our contribution is that, in a clinical setting, propofol exerts a greater effect on the promoter region of *HO-1* than does isoflurane.

In conclusion, our findings suggest that an effect of propofol in this setting is an anti-inflammatory or an inflammation-delaying action. If genuine, these may be valuable to patients undergoing CPB, although further studies with larger numbers of patients and in different clinical settings is warranted. □

The authors declare no conflicts of interest.

References

1 Bernhard M, Schultz K, Hofer S *et al*. MIF and markers of the inflammatory response following cardiac surgery under extracorporeal circulation. *Appl Cardiopulm Pathophysiol* 2010; **14**: 115–23.

2 Day JR, Taylor KM. The systemic inflammatory response syndrome and cardiopulmonary bypass. *Int J Surg* 2005; **3** (2): 129–40.

3 Durandy Y. Minimizing systemic inflammation during cardiopulmonary bypass in the pediatric population. *Artif Organs* 2014; **38** (1): 11–8.

4 Warren OJ, Smith AJ, Alexiou C *et al*. The inflammatory response to cardiopulmonary bypass: part 1 – mechanisms of pathogenesis. *J Cardiothorac Vasc Anesth* 2009; **23** (2): 223–31.

5 Li S, Price R, Phiroz D, Swan K, Crane TA. Systemic inflammatory response during cardiopulmonary bypass and strategies. *J Extra Corpor Technol* 2005; **37** (2): 180–8.

6 Schneemilch CE, Hachenberg T, Ansorge S, Ittenson A, Bank U. Effects of different anaesthetic agents on immune cell function *in vitro*. *Eur J Anaesthesiol* 2005; **22** (8): 616–23.

7 Zhou R, Yang Z, Tang X, Tan Y, Wu X, Liu F. Propofol protects against focal cerebral ischemia via inhibition of microglia-mediated proinflammatory cytokines in a rat model of experimental stroke. *PLoS One* 2013; 8; **9** (12): e82729.

8 Li Volti G, Basile F, Murabito P *et al*. Antioxidant properties of anesthetics: the biochemist, the surgeon and the anesthetist. *Clin Ter* 2008; **159** (6): 463–9.

9 Jaffer U, Wade RG, Gourlay T. Cytokines in the systemic inflammatory response syndrome: a review. *HSR Proc Intensive Care Cardiovasc Anesth* 2010; **2** (3): 161–75.

10 Mazoti M, Braz M, de Assis G *et al*. Comparison of inflammatory cytokine profiles in plasma of patients undergoing otorhinological surgery with propofol or isoflurane anesthesia. *Inflamm Res* 2013; **62** (10): 879–85.

11 Murphy GS, Szokol JW, Marymont JH, Avram MJ, Vender JS. The effects of morphine and fentanyl on the inflammatory response to cardiopulmonary bypass in patients undergoing elective coronary artery bypass graft surgery. *Anesth Analg* 2007; **104** (6): 1334–42.

12 Asimakopoulos G, Kohn A, Stefanou DC, Haskard DO, Landis RC, Taylor KM. Leukocyte integrin expression in patients undergoing cardiopulmonary bypass. *Ann Thorac Surg* 2000; **69** (4): 1192–7.

13 Wu Q, Purusram G, Wang H *et al*. The efficacy of parecoxib on systemic inflammatory response associated with cardiopulmonary bypass during cardiac surgery. *Br J Clin Pharmacol* 2013; **75** (3): 769–78.

14 Greer SN, Metcalf JL, Wang Y, Ohh M. The updated biology of hypoxia-inducible factor. *EMBO J* 2012; **31** (11): 2448–60.

15 Hölscher M, Schäfer K, Krull S *et al*. Unfavourable consequences of chronic cardiac HIF-1α stabilization. *Cardiovasc Res* 2012; **94** (1): 77–86.

16 Chen HB, Wang L, Jiang JF. Re-analysis of expression profiles for revealing new potential candidate genes of heart failure. *Eur Rev Med Pharmacol Sci* 2013, **17** (7): 903–11.

17 Idriss NK, Blann AD, Lip GY. Hemoxygenase-1 in cardiovascular disease. *J Am Coll Cardiol* 2008; **52** (12): 971–8.

18 Lavrovsky Y, Schwartzman ML, Levere RD, Kappas A, Abraham NG. Identification of binding sites for transcription factors NF-kappa B and AP-2 in the promoter region of the human heme oxygenase 1 gene. *Proc Natl Acad Sci USA* 1994; **91** (13): 5987–91.

19 Chen YH, Lin SJ, Lin MW *et al*. Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. *Hum Genet* 2002; **111** (1): 1–8.

20 Dick P, Schillinger M, Minar E *et al.* Haem oxygenase-1 genotype and cardiovascular adverse events in patients with peripheral artery disease. *Eur J Clin Invest* 2005; **35** (12): 731–7.

21 Schmidt R, Tritschler E, Hoetzel A *et al*. Heme oxygenase-1 induction by the clinically used anesthetic isoflurane protects rat livers from ischemia/reperfusion injury. *Ann Surg* 2007; **245** (6): 931–42.

22 Gold MI, Sacks DJ, Grosnoff DB, Herrington CA. Comparison of propofol with thiopental and isoflurane for induction and maintenance of general anesthesia. *J Clin Anesth* 1989: **1** (4): 272–6.

23 Zgola K, Kulakowski P, Czepiel A *et al*. Haemodynamic effects of etomidate, propofol and electrical shock in patients undergoing implantable cardioverter-defibrillator testing. *Kardiol Pol* 2014; **72** (8): 707–15.

24 Korttila K, Ostman P, Faure E *et al.* Randomized comparison of recovery after propofol-nitrous oxide versus thiopentone-isoflurane-nitrous oxide anaesthesia in patients undergoing ambulatory surgery. *Acta Anaesthesiol Scand* 1990; **34** (5): 400–3.

25 Jeong CW, Ju J, Lee DW, Lee SH, Yoon MH. Lipid-emulsion propofol less attenuates the regulation of body temperature than micro-emulsion propofol or sevoflurane in the elderly. *Yonsei Med J* 2012; **53** (1): 198–203.

26 Liu TC. Influence of propofol, isoflurane and enflurance on levels of serum interleukin-8 and interleukin-10 in cancer patients. *Asian Pac J Cancer Prev* 2014; **15** (16): 6703–7.

27 Yoo YC, Shim JK, Song Y, Yang SY, Kwak YL. Anesthetics influence the incidence of acute kidney injury following valvular heart surgery. *Kidney Int* 2014; **86** (2): 414–22.

28 Ke JJ, Zhan J, Feng XB, Wu Y, Rao Y, Wang YL. A comparison of the effect of total intravenous anaesthesia with propofol and remifentanil and inhalational anaesthesia with isoflurane on the release of pro- and anti-inflammatory cytokines in patients undergoing open cholecystectomy. *Anaesth Intensive Care* 2008; **36** (1): 74–8.

29 Corcoran TB, Engel A, Sakamoto H, O'Shea A, O'Callaghan-Enright S, Shorten GD. The effects of propofol on neutrophil function, lipid peroxidation and inflammatory response during elective coronary artery bypass grafting in patients with impaired ventricular function. *Br J Anaesth* 2006; **97** (6): 825–31.

30 Baki ED, Aldemir M, Kokulu S *et al*. Comparison of the effects of desflurane and propofol anaesthesia on the inflammatory response and s100 protein during coronary artery bypass grafting. *Inflammation* 2013; **36** (6): 1327–33.

31 Hambsch J, Osmancik P, Bocsi J, Schneider P, Tárnok A. Neutrophil adhesion molecule expression and serum concentration of soluble adhesion molecules during and after pediatric cardiovascular surgery with or without cardiopulmonary bypass. *Anesthesiology* 2002; **96** (5): 1078–85.

32 Pa nik J, Baj Z, Moll JA *et al*. Effects of cardiopulmonary bypass on the expression of adhesive molecules in children undergoing cardiac surgery due to congenital heart disorders. *Kardiol Pol* 2004; **60** (2): 123–31. Discussion 132.

33 Tanaka T, Takabuchi S, Nishi K *et al*. The intravenous anesthetic propofol inhibits lipopolysaccharide-induced hypoxia-inducible factor 1 activation and suppresses the glucose metabolism in macrophages. *J Anesth* 2010; **24** (1): 54–60.

34 Yeh CH, Cho W, So EC *et al.* Propofol inhibits lipopoly-saccharide-induced lung epithelial cell injury by reducing

hypoxia-inducible factor-1alpha expression. *Br J Anaesth* 2011; **106** (4): 590–9.

35 Idriss NK, Lip GY, Balakrishnan B, Jaumdally R, Boos CJ, Blann AD. Plasma haemoxygenase-1 in coronary artery disease. A comparison with angiogenin, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 and vascular endothelial growth factor. *Thromb Haemost* 2010; **104** (5): 1029–37.

36 Zheng H, Ma HP, Wang J, Ma M. Preoperative HO-1 levels as prognostic factor for adverse cardiac events in elder patients undergoing non-cardiac surgery. *PLoS One* 2013; **8** (3): e58567.

37 Hoetzel A, Schmidt R. Regulatory role of anesthetics on heme-oxygenase-1. *Curr Drug Targets* 2010; **11** (12): 1495–503.

38 Xia WF, Liu Y, Zhou QS, Tang QZ, Zou HD. Comparison of the effects of propofol and midazolam on inflammation and oxidase stress in children with congenital heart disease undergoing cardiac surgery. *Yonsei Med J* 2011; **52** (2): 326–32.

39 Gu J, Chi M, Sun X *et al*. Propofol-induced protection of SH-SY5Y cells against hydrogen peroxide is associated with the HO-1 via the ERK pathway. *Int J Med Sci* 2013; **10** (5): 599–606.

40 Xu J, Li H, Irwin MG *et al*. Propofol ameliorates hyperglycemia-induced cardiac hypertrophy and dysfunction via heme oxygenase-1/signal transducer and activator of transcription 3 signaling pathway in rats. *Crit Care Med* 2014; **42** (8): e583–94.