Intensive Care Med (2008) 34:1216–1223
DOI 10.1007/s00134-008-1042-7

ORIGINAL

Barry Dixon
Duncan J. Campbell
John D. Santamaria

Elevated pulmonary dead space and coagulation abnormalities suggest lung microvascular thrombosis in patients undergoing cardiac surgery

Received: 18 October 2007
Accepted: 30 December 2007
Published online: 27 February 2008

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-008-1042-7) contains supplementary material, which is available to authorized users.

This article is discussed in the editorial available at: http://dx.doi.org/10.1007/s00134-008-1043-6.

The study was registered with the Australian Clinical Trials Registry No. 12605000133639.
URL: http://www.actr.org.au/.

B. Dixon (✉) · J. D. Santamaria
St. Vincent's Hospital, Intensive Care Unit, Melbourne, 3065, Victoria, Australia
e-mail: barry.dixon@svhm.org.au
Tel.: +61-3-92884488
Fax: +61-3-92884487

D. J. Campbell
St. Vincent's Institute of Medical Research, Melbourne, 3065, Victoria, Australia

Abstract *Objective:* Inflammation has been shown to trigger microvascular thrombosis. Patients undergoing cardiac surgery sustain significant inflammatory insults to the lungs and in addition are routinely given anti-fibrinolytic agents to promote thrombosis. In view of these risk factors we investigated if evidence of pulmonary microvascular thrombosis occurs following cardiac surgery and, if so, whether a pre-operative heparin infusion may limit this. *Design:* Double-blind randomised controlled trial. *Setting:* Tertiary university affiliated hospital. *Patients:* Twenty patients undergoing elective cardiac surgery. *Interventions:* Patients were randomised to receive a pre-operative heparin infusion or placebo. All patients were administered aprotinin. *Measurements and results:* Pulmonary microvascular obstruction was estimated by measuring the alveolar dead-space fraction. Pulmonary coagulation activation was estimated by measuring the ratio of prothrombin fragment levels in radial and pulmonary arterial blood. Systemic tissue plasminogen activator (t-PA) levels were also assessed. In the placebo group cardiac surgery triggered increased alveolar dead-space fraction levels and the onset of prothrombin fragment production in the pulmonary circulation. Administration of pre-operative heparin was associated with a lower alveolar dead-space fraction ($p < 0.05$) and reduced prothrombin fragment production in the pulmonary circulation ($p < 0.05$). Pre-operative heparin also increased baseline t-PA levels ($p < 0.05$). *Conclusion:* The changes in the alveolar dead-space fraction and pulmonary coagulation activation suggest that pulmonary microvascular thrombosis develops during cardiac surgery and this may be limited by a pre-operative heparin infusion.

Keywords Cardiopulmonary bypass · Coagulation · Fibrinolysis · Inflammation · Thrombosis

Introduction

In patients undergoing cardiac surgery a range of inflammatory insults contribute to post-operative lung injury [1, 2]. Inflammatory insults include lung ischaemia sustained during cardiopulmonary bypass (CPB), surgical trauma and contact of blood with the foreign surface of the CPB circuit [3–7]. In other inflammatory conditions, such as sepsis, microvascular thrombosis has been shown to be a mechanism of organ injury [8, 9]. Stimulation of microvascular endothelial cells by inflammatory mediators is the initial process that gives rise to microvascular thrombosis [10]. In response, endothelial cells express platelet and white cell ligands, such as vascular cell adhesion molecule-1 (VCAM-1) [11–13]. Within minutes platelets and white cells adhere to the endothelium forming cellular aggregates. The endothelium and aggregated cells, in turn, express tissue factor, which triggers

coagulation activation [8]. These responses result in fibrin deposition and microvascular thrombosis [14–18]. In patients undergoing cardiac surgery the risk of developing microvascular thrombosis may be further increased by the routine use of anti-fibrinolytic agents to promote thrombosis [19, 20].

Case reports have demonstrated microvascular thrombi in the lungs, heart and kidneys of patients that died following cardiac surgery [21–25]. In a case series of nine patients that developed acute post-operative pulmonary hypertension, multiple microvascular thrombi were demonstrated in the lungs [25].

In this study we sought to assess if a pre-operative heparin infusion limited evidence of pulmonary microvascular thrombosis. Pulmonary microvascular thrombosis was estimated by measuring the alveolar dead-space fraction (the alveolar dead space to tidal volume ratio) [26] and the extent of coagulation activation in the pulmonary circulation was estimated by measuring the ratio of prothrombin fragment levels in radial and pulmonary arterial blood.

Materials and methods

Subjects

We studied patients undergoing elective coronary artery bypass grafting (CABG) with CPB. Patients were excluded if they had had previous CABG or required a surgical intervention in addition to CABG, a creatinine level greater than 200 umol/l or age greater than 85 years. The study was approved by the St. Vincent's Hospital Human Research Ethics Committee. All patients gave written informed consent before participation in the study.

Interventions

We undertook a double-blind placebo-controlled trial. Patients were randomised (computer-generated blocks of four) to a continuous pre-operative infusion of heparin or placebo. The infusion bags (500 ml of 5% glucose) and rates of infusion were identical in both groups. The pre-operative heparin group had 25,000 units (U) of heparin (Porcine Heparin Sodium, Pharmacia, Melbourne, Australia) added to the bag. Drug preparation was performed by nurses not involved with data collection. The infusion commenced with a bolus of 100 ml of fluid (5000 U of heparin) over 30 min, and was then continued at 0.36 ml/kg h^{-1} (18 U of heparin/kg h^{-1}). The infusion commenced on average 10 h before surgery and was continued until the intra-operative heparin bolus – given just before commencement of CPB.

Data collection

The alveolar dead-space fraction, the alveolar arterial (A-a) oxygen gradient, lactate, Hb and haemodynamic variables were measured following anaesthetic induction, following sternotomy and at 0, 1, 2, 3 and 4 h post-CPB. At the same time points blood was aspirated from the distal port of the pulmonary artery catheter and then immediately from the radial arterial line for prothrombin fragments, blood gas, white cell and platelet levels. The ratio of prothrombin fragment levels in radial and pulmonary arterial blood was calculated. A ratio greater than 1 suggests coagulation activation in the pulmonary circulation. Tissue plasminogen activator, s-VCAM-1 and troponin I levels were sampled at the same time points from the radial arterial line. The ventilator and ventilator settings were standardised for all measurements. The alveolar dead space was measured using the Cosmo Plus Respironics monitor (Novametrix Medical Systems, Connecticut). The A-a gradient was calculated using standard formulae. The left ventricular (LV) ejection fraction was graded by intra-operative trans-oesophageal echocardiography, where severe dysfunction represents a ejection fraction $< 30\%$, moderate 30–44%, mild 45–54% and normal $\geq 55\%$. Haemodynamic variables were measured with a pulmonary artery catheter using standard techniques. Automated laboratory analyses of Hb, white cell and platelet levels were undertaken. Prothrombin fragments, t-PA, s-VCAM-1 and troponin-I levels were assayed by enzyme-linked immunoassays. (For more details regarding data collection see the ESM.)

Anaesthesia and surgical management

Patients were administered 2 million units IV aprotinin following induction followed by 0.5 million U/h for the duration of the operation. An additional 2 million units was added to the pump prime. All patients were hep-

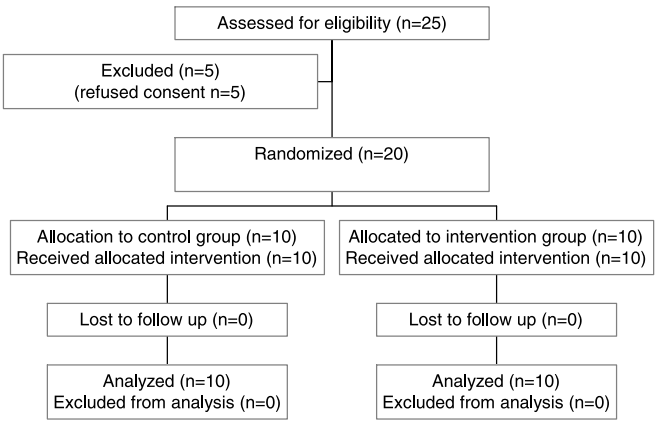

Fig. 1 The Consort flow diagram showing participant progress

arinised just before commencement of CPB (initial dose ~ 300 U/kg) to maintain an activated clotting time (using kaolin) above 400 s. (For more details see the ESM.)

Statistical analysis

The study was powered to demonstrate a 30% difference in the alveolar dead-space fraction, with 80% power at a significance level of 0.05. Fisher's exact test compared categorical variables. Student's *t*-test compared normally distributed variables and the Mann–Whitney test non-normally distributed variables. Repeated measures analysis of variance (ANOVA) was used to compare variables repeatedly measured over time. Statistical analysis was performed with Statview (SAS Institute, Cary, NC, USA).

Results

The Consort flow diagram presents participant progress (Fig. 1). Twenty patients were studied, 10 received pre-operative heparin and 10 placebo. The baseline and operative characteristics for the two groups were similar (Tables 1, 2). The APTT level at anaesthesia induction was significantly higher in the heparin group (126 vs. 37 s, $p < 0.0001$). One patient in the pre-operative heparin group died from acute heart failure, as a result of graft failure, on the second post-operative day. Operative characteristics, including CPB and cross-clamp times, number of grafts and intra-operative heparin dose, were similar between groups (Table 2).

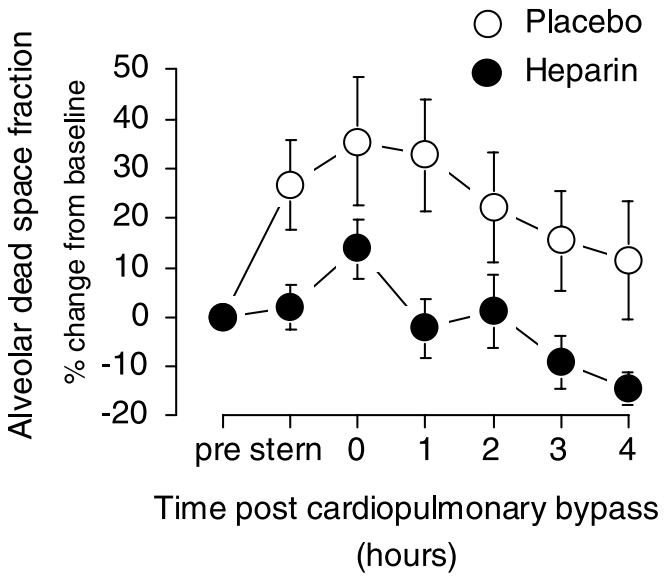

Fig. 2 The alveolar dead-space fraction in patients undergoing cardiac surgery. *Stern* indicates post-sternotomy. Data shown as mean ± SEM. The alveolar dead-space fraction was lower in the pre-operative heparin group ($p < 0.05$, repeated-measures ANOVA comparison of group and also group time interaction)

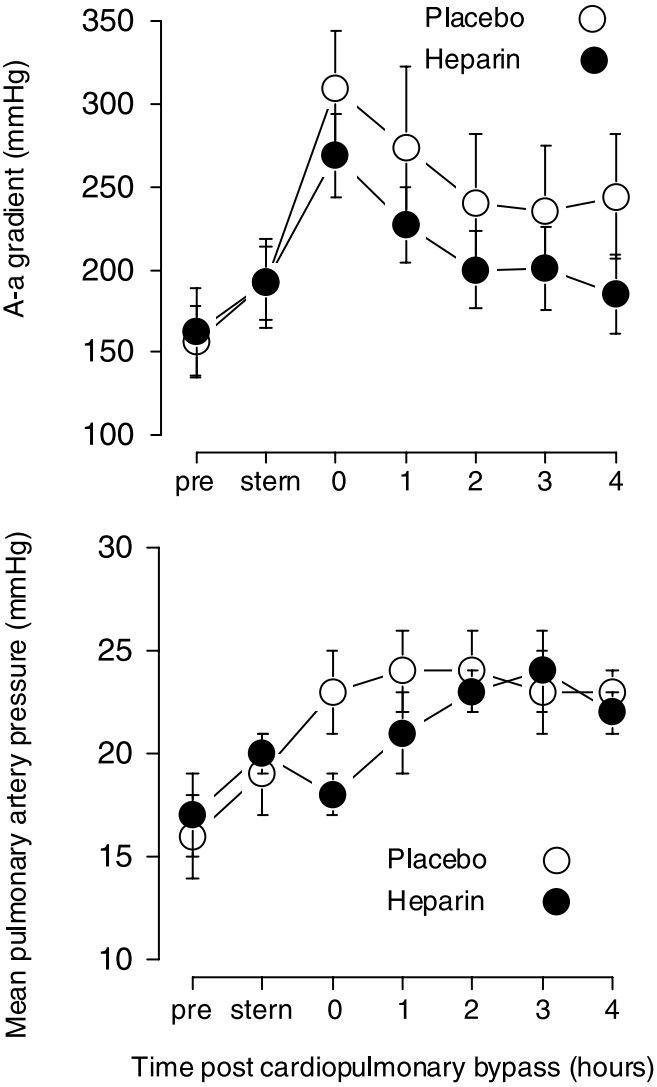

Fig. 3 The alveolar to arterial oxygen gradient ($A-a$) and mean pulmonary artery pressure levels in patients undergoing cardiac surgery. *Stern* indicates post-sternotomy. Data shown as mean ± SEM. There were no significant differences between groups (repeated-measures ANOVA)

Pulmonary responses

At baseline the alveolar dead-space fraction was 0.22 in both groups. In the placebo group the alveolar dead-space fraction increased markedly following cardiac surgery. The alveolar dead-space fraction increased 27% following sternotomy and increased further to 35% above baseline levels at the end of CPB; thereafter, levels fell but were still

Table 1 Baseline characteristics. *ACE*, angiotensin converting enzyme; *CCS*, Canadian Cardiovascular Society; *LV*, left ventricle; *t-PA*, tissue plasminogen activator; *PTF*, prothrombin fragments; *s-VCAM-1*, soluble vascular cell adhesion molecule

Characteristic	Heparin	Placebo	Significance (p)
Age (years)	63 ± 12	61 ± 8	0.6
Male (%)	100	80	0.5
Ever smoked (%)	70	80	0.6
Pack years	24 ± 22	30 ± 40	0.7
Diabetes (%)	30	40	0.6
Hypertension (%)	50	80	0.4
Statin (%)	90	100	1.0
ACE inhibitor (%)	60	50	0.7
Aspirin stopped (%) [a]	50	80	0.4
CCS angina class	2.2 ± 0.6	1.7 ± 0.7	0.10
Infusion hours	9.5 ± 1.2	9.8 ± 1.3	0.6
LV function (%)			
Normal	70	80	
Mild impairment	20	20	
Moderate impairment	0	0	
Severe impairment	10	0	0.2
t-PA (ng/ml)	11 (8–15)	7 (6–9)	< 0.05
PTF (pmol/l)	120 ± 56	189 ± 57	0.01
s-VCAM-1 (ng/ml)	321 (297–403)	330 (291–389)	0.7

Data shown as mean $\pm$ SD or median (interquartile range); [a] Aspirin stopped 7 days before surgery

Table 2 Operative characteristics. *CPB*, cardiopulmonary bypass; *ACT*, activated clotting time

Characteristic	Heparin	Placebo	Significance (p)
CPB time (min)	95 ± 19	101 ± 16	0.4
Cross-clamp time (min)	73 ± 21	81 ± 14	0.3
Total grafts	3.2 ± 0.6	3.3 ± 0.7	0.7
Arterial grafts	2.5 ± 0.7	2.8 ± 0.9	0.4
Vein grafts	0.7 ± 1.2	0.5 ± 0.9	0.7
Minimum temperature (°C)	32.7 ± 0.5	32.4 ± 0.6	0.3
Heparin at onset CPB (units/kg)	314 ± 47	317 ± 28	0.9
Pre-ACT (s)	174 ± 32	153 ± 22	0.1
Maximum ACT during CPB (s)	826 ± 150	852 ± 86	0.6
Post-CPB ACT (s)	140 ± 15	137 ± 14	0.6

Data shown as mean $\pm$ SD

Table 3 Post-operative characteristics. *ICU*, intensive care unit; *MAP*, mean arterial pressure; *CI*, cardiac index

Characteristic	Heparin	Placebo	Significance (p)
ICU stay (h)	32 ± 26	29 ± 25	0.8
Hospital stay (days)	6.8 ± 2.8	7.6 ± 1.4	0.5
Ventilation (h)	12.4 ± 4.1	15.1 ± 4.9	0.2
Adrenaline (%)	20	10	0.5
Noradrenaline (%)	30	20	0.6
MAP (mmHg) [a]	85 ± 7	84 ± 7	0.7
CI (l/min m^{-2}) [a]	2.7 ± 0.3	2.9 ± 0.5	0.4
Lactate (mmol/l) [a]	1.8 ± 0.8	2.1 ± 0.8	0.3
Haemoglobin (g/l) [a]	94 ± 10	97 ± 8	0.5
Chest tube blood loss at 4 h post-CPB (ml)	255 (210–320)	170 (100–205)	< 0.05
Packed cells transfused	0.7 ± 1.6	0.2 ± 0.6	0.4

Data shown as mean $\pm$ SD or median (interquartile range); [a] The summary value represents the average value for the 0-, 1-, 2-, 3- and 4-h time points post-CPB

above baseline levels at 4 h post-CPB. Administration of pre-operative heparin was associated with lower alveolar dead-space fraction levels ($p < 0.05$, comparison of group and also group time interaction; Fig. 2). The A-a gradient increased to a similar extent in both groups following cardiac surgery (Fig. 3). The mean pulmonary artery pressure was similar in both groups following cardiac surgery CPB (Fig. 3).

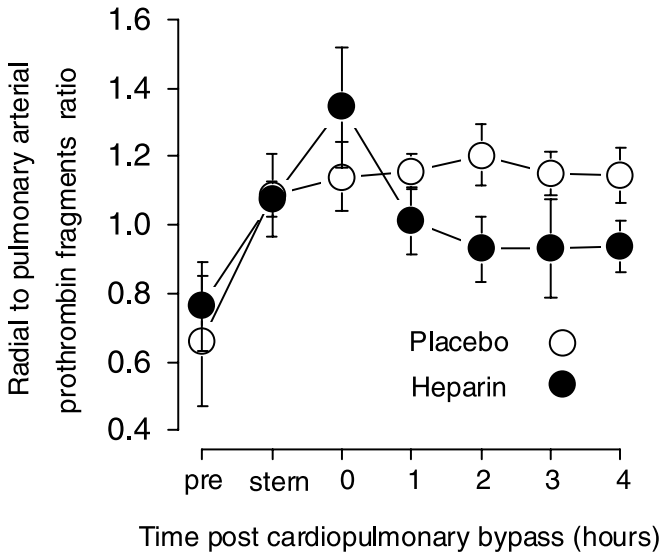

Fig. 4 Radial to pulmonary arterial prothrombin fragment ratio in patients undergoing cardiac surgery. *Stern* indicates post-sternotomy. Data shown as mean ± SEM. Pre-operative heparin reduced pulmonary prothrombin fragment production post-CPB ($p < 0.05$, repeated-measures ANOVA comparison between groups from 1 h post-CPB)

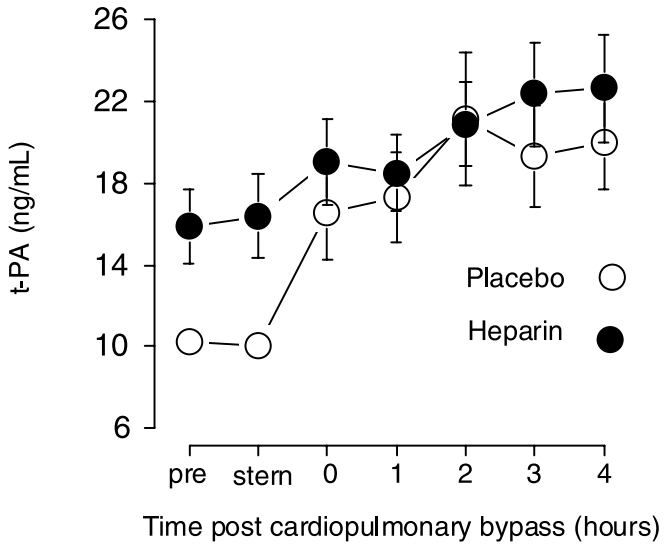

Fig. 5 Tissue plasminogen activator (*t-PA*) levels in patients undergoing cardiac surgery. *Stern* indicates post-sternotomy. Data shown as mean ± SEM. Levels were higher in the pre-operative heparin group at time points prior to cardiopulmonary bypass ($p < 0.05$)

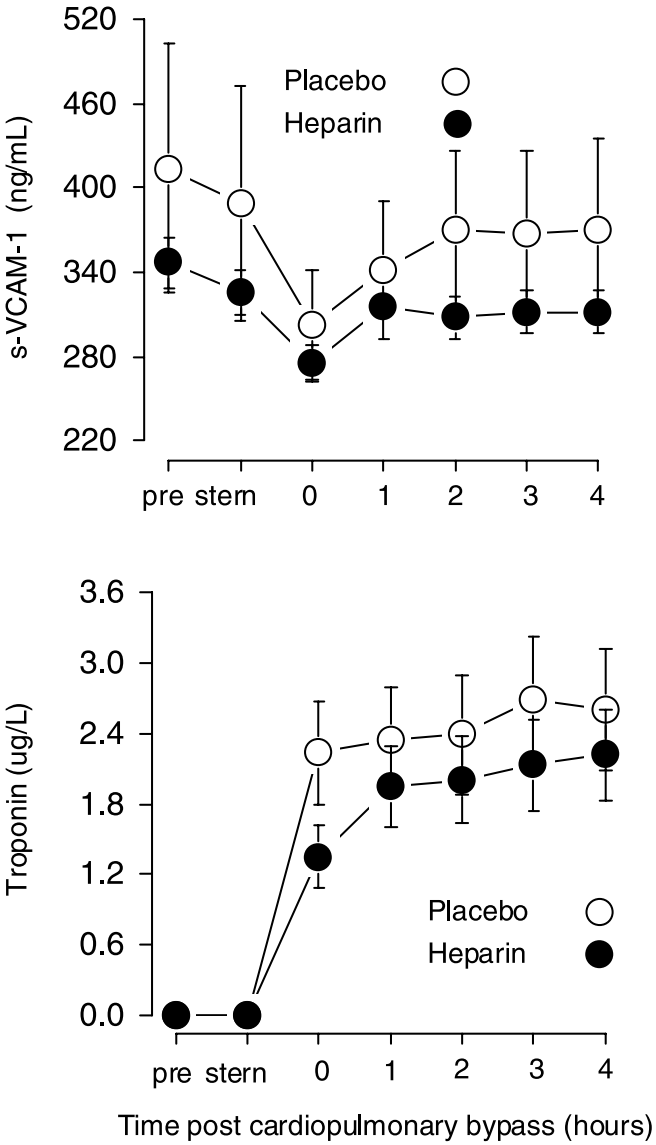

Fig. 6 Soluble vascular cell adhesion molecule-1 (*s-VCAM-1*) and troponin-I levels in patients undergoing cardiac surgery. *Stern* indicates (post-sternotomy). There were no significant differences between groups (repeated-measures ANOVA)

Coagulation activation in the pulmonary circulation

The ratio of radial to pulmonary arterial blood prothrombin fragment levels at baseline was less than 1 in both groups. At sternotomy and at the end of CPB, the ratio was greater than 1 in both groups; thereafter, the ratio remained greater than 1 in the placebo group, but fell below 1 in the pre-operative heparin group ($p < 0.05$, comparison of group; Fig. 4). Systemic prothrombin fragment levels were also reduced at baseline in the pre-operative heparin group ($p = 0.01$; Table 1).

Tissue plasminogen activator

Blood t-PA levels were significantly higher at both baseline ($p < 0.05$; Table 1) and post-sternotomy ($p = 0.01$) in the pre-operative heparin group. Subsequent CPB levels increased about twofold in both groups and remained ele-

vated thereafter. The levels reached were similar in both groups post-CPB (Fig. 5).

Vascular cell adhesion molecule-1 and troponin-I levels

Blood s-VCAM-1 levels were similar in both groups throughout the study period (Fig. 6). Troponin-I levels were initially lower at the end of CPB, in the pre-operative heparin group, and thereafter levels were similar (Fig. 6).

Pulmonary white cell and platelet retention

The changes in the radial to pulmonary arterial platelet and white cell ratios were similar in both groups. We did not find significant evidence of pulmonary platelet or white cell retention.

Post-operative characteristics

Post-operative characteristics, including hours of mechanical ventilation, intensive care unit and hospital length of stays, haemodynamic characteristics, inotrope use, Hb and lactate levels, were similar between groups. Chest-drain blood loss at 4 h was greater in the pre-operative heparin group, but transfusion requirements were similar between groups (Table 3).

Discussion

Our study found evidence suggesting that pulmonary microvascular thrombosis occurs during cardiac surgery. Firstly, in the placebo group we found that the alveolar dead-space fraction increased by 35% compared with baseline levels following cardiac surgery (a finding consistent with reduced alveolar perfusion). Secondly, we demonstrated that cardiac surgery triggered coagulation activation in the pulmonary circulation. Finally, we demonstrated that a pre-operative heparin infusion was associated with reduced alveolar dead-space fraction levels and reduced coagulation activation in the pulmonary circulation.

This effect of pre-operative heparin in limiting coagulation activation in the pulmonary circulation may appear surprising in view of the fact that both groups were administered 300 U/kg of heparin just before commencement of CPB; however, a number of the anti-coagulant actions of heparin manifest through pathways requiring protein synthesis; hence, these actions peak some hours after heparin administration. The addition of a pre-operative heparin infusion to the standard intra-operative bolus of heparin may theoretically therefore enhance heparin's anti-thrombotic properties during cardiac surgery. These anti-coagulant actions include endothelial expression of heparan sulfate [27], endothelial and platelet secretion of tissue factor pathway inhibitor (TFPI) and inhibition of endothelial and monocyte expression of tissue factor [28, 29]. Our finding that post-operative chest tube drainage was significantly greater in the pre-operative heparin group also supports this contention.

Heparin has also previously been shown to trigger increased endothelial expression of t-PA [30]. As expected, therefore, t-PA levels were significantly increased before CPB in the pre-operative heparin group. Following the intra-operative heparin bolus levels increased in both groups to a similar extent. Enhancement of fibrinolysis may also be a mechanism by which pre-operative heparin limited the increase in the alveolar dead-space fraction. Pre-operative heparin had no effect on s-VCAM-1 levels or the extent of white cell or platelet retention in the lungs. Pre-operative heparin did not significantly improve the A-a gradient, mean pulmonary artery pressure levels or troponin I levels.

Our finding that cardiac surgery was associated with a marked increase in the alveolar dead-space fraction is consistent with other forms of acute inflammatory lung injury, such as the acute respiratory distress syndrome (ARDS). A recent study of patients presenting to the emergency department with ARDS found that the extent of the increase in the alveolar dead space was an independent predictor of death [31].

The administration of aprotinin may have played a role in our finding of evidence of pulmonary microvascular thrombosis. Case reports have demonstrated an association between aprotinin and histological evidence of microvascular thrombosis [21–25]. In addition, aprotinin has been implicated in the development of multi-organ failure following cardiac surgery [19, 20].

Potential limitations

The major limitations of our study were the indirect methods used to assess evidence of pulmonary microvascular thrombosis. Factors other than microvascular obstruction may increase the alveolar dead-space fraction. Alveolar blood flow may fall due to low blood pressure or a poor cardiac output [32]. Blood pressure and cardiac output levels were, however, adequate and equivalent in both groups. Variations in ventilation parameters, such as tidal volume and respiratory rate, may also increase the alveolar dead-space fraction [33]. The ventilation parameters were, however, kept constant throughout the study period.

Atelectasis may also contribute to an increase in the alveolar dead space through high V/Q mismatch. Our interpretation of the changes in the ratio of radial to pulmonary arterial prothrombin fragment levels may be

questioned. Our interpretation is supported by a study of patients undergoing cardiac surgery that demonstrated increased intravascular fibrin formation following reperfusion of the lungs and heart [34], and also by animal models of CPB and pulmonary ischaemic-reperfusion injury, which demonstrated pulmonary microvascular thrombosis and beneficial outcomes associated with anti-coagulants [14–17].

The major implications of our study are that microvascular thrombosis may be a mechanism of lung injury in patients undergoing cardiac surgery, and that this may be limited by a pre-operative heparin infusion. Further work, however, is required to establish this.

Acknowledgements. This study was supported by the St. Vincent's Hospital Research Endowment Fund, The Intensive Care Foundation and by departmental funds.

References

1. Ng CS, Wan S, Yim AP, Arifi AA (2002) Pulmonary dysfunction after cardiac surgery. Chest 121:1269–1277
2. Massoudy P, Zahler S, Becker BF, Braun SL, Barankay A, Meisner H (2001) Evidence for inflammatory responses of the lungs during coronary artery bypass grafting with cardiopulmonary bypass. Chest 119:31–36
3. Serraf A, Robotin M, Bonnet N, Detruit H, Baudet B, Mazmanian MG, Herve P, Planche C (1997) Alteration of the neonatal pulmonary physiology after total cardiopulmonary bypass. J Thorac Cardiovasc Surg 114:1061–1069
4. Schlensak C, Doenst T, Preusser S, Wunderlich M, Kleinschmidt M, Beyersdorf F (2001) Bronchial artery perfusion during cardiopulmonary bypass does not prevent ischemia of the lung in piglets: assessment of bronchial artery blood flow with fluorescent microspheres. Eur J Cardiothorac Surg 19:326–331
5. Chai PJ, Williamson JA, Lodge AJ, Daggett CW, Scarborough JE, Meliones JN, Cheifetz IM, Jaggers JJ, Ungerleider RM (1999) Effects of ischemia on pulmonary dysfunction after cardiopulmonary bypass. Ann Thorac Surg 67:731–735
6. Suzuki T, Ito T, Kashima I, Teruya K, Fukuda T (2001) Continuous perfusion of pulmonary arteries during total cardiopulmonary bypass favorably affects levels of circulating adhesion molecules and lung function. J Thorac Cardiovasc Surg 122:242–248
7. Wan S, LeClerc JL, Vincent JL (1997) Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. Chest 112:676–692
8. Dixon B (2004) The role of microvascular thrombosis in sepsis. Anaesth Intensive Care 32:619–629
9. Sapru A, Wiemels JL, Witte JS, Ware LB, Matthay MA (2006) Acute lung injury and the coagulation pathway: potential role of gene polymorphisms in the protein C and fibrinolytic pathways. Intensive Care Med 32:1293–1303
10. Beck G, Habicht GS, Benach JL, Miller F (1986) Interleukin 1: a common endogenous mediator of inflammation and the local Shwartzman reaction. J Immunol 136:3025–3031
11. Dosquet C, Weill D, Wautier JL (1995) Cytokines and thrombosis. J Cardiovasc Pharmacol Suppl 25(2):S13–S19
12. Blume ED, Nelson DP, Gauvreau K, Walsh AZ, Plumb C, Neufeld EJ, Hickey PR, Mayer JE, Newburger JW (1997) Soluble adhesion molecules in infants and children undergoing cardiopulmonary bypass. Circulation 96:II-352–357
13. Massoudy P, Zahler Sea, Becker BF, Braun SL, Barankay A, Richter JA, Meisner H (1999) Significant leukocyte and platelet retention during pulmonary passage after declamping of the aorta in CABG patients. Eur J Med Res 4:178–182
14. Tanaka K (2001) Specific inhibition of thrombin activity during cardiopulmonary bypass reduces ischemia-reperfusion injury of the lung. Fukuoka Igaku Zasshi 92:7–20
15. Okada K, Fujita T, Minamoto K, Liao H, Naka Y, Pinsky DJ (2000) Potentiation of endogenous fibrinolysis and rescue from lung ischemia/reperfusion injury in interleukin (IL)-10-reconstituted IL-10 null mice. J Biol Chem 275:21468–21476
16. Pinsky DJ, Liao H, Lawson CA, Yan SF, Chen J, Carmeliet P, Loskutoff DJ, Stern DM (1998) Coordinated induction of plasminogen activator inhibitor-1 (PAI-1) and inhibition of plasminogen activator gene expression by hypoxia promotes pulmonary vascular fibrin deposition. J Clin Invest 102:919–928
17. Lawson CA, Yan SD, Yan SF, Liao H, Zhou YS, Sobel J, Kisiel W, Stern DM, Pinsky DJ (1997) Monocytes and tissue factor promote thrombosis in a murine model of oxygen deprivation. J Clin Invest 99:1729–1738
18. Argenbright LW, Barton RW (1992) Interactions of leukocyte integrins with intercellular adhesion molecule 1 in the production of inflammatory vascular injury in vivo. The Shwartzman reaction revisited. J Clin Invest 89:259–272
19. Mangano DT (2002) Aspirin and mortality from coronary bypass surgery. N Engl J Med 347:1309–1317
20. Mangano DT, Tudor IC, Dietzel C (2006) The risk associated with aprotinin in cardiac surgery. N Engl J Med 354:353–365
21. Saffitz JE, Stahl DJ, Sundt TM, Wareing TH, Kouchoukos NT (1993) Disseminated intravascular coagulation after administration of aprotinin in combination with deep hypothermic circulatory arrest. Am J Cardiol 72:1080–1082
22. Sundt TM III, Kouchoukos NT, Saffitz JE, Murphy SF, Wareing TH, Stahl DJ (1993) Renal dysfunction and intravascular coagulation with aprotinin and hypothermic circulatory arrest. Ann Thorac Surg 55:1418–1424
23. Blaisdell FW, Lim RC Jr, Amberg JR, Choy SH, Hall AD, Thomas AN (1966) Pulmonary microembolism. A cause of morbidity and death after major vascular surgery. Arch Surg 93:776–786
24. Gregoric ID, Patel V, Radovancevic R, Bracey AW, Radovancevic B, Frazier OH (2005) Pulmonary microthrombi during left ventricular assist device implantation. Tex Heart Inst J 32:228–231
25. Cooper JR Jr, Abrams J, Frazier OH, Radovancevic R, Radovancevic B, Bracey AW, Kindo MJ, Gregoric ID (2006) Fatal pulmonary microthrombi during surgical therapy for end-stage heart failure: possible association with antifibrinolytic therapy. J Thorac Cardiovasc Surg 131:963–968
26. Severinghaus JW, Stupfel M (1957) Alveolar dead space as an index of distribution of blood flow in pulmonary capillaries. J Appl Physiol 10:335–348

27. Cadroy Y, Gaspin D, Dupouy D, Lormeau JC, Boneu B, Sie P (1996) Heparin reverses the procoagulant properties of stimulated endothelial cells. Thromb Haemost 75:190–195
28. Gori AM, Pepe G, Attanasio M, Falciani M, Abbate R, Prisco D, Fedi S, Giusti B, Brunelli T, Comeglio P, Gensini GF, Neri Serneri GG (1999) Tissue factor reduction and tissue factor pathway inhibitor release after heparin administration. Thromb Haemost 81:589–593
29. Pepe G, Giusti B, Attanasio M, Gori AM, Comeglio P, Martini F, Gensini G, Abbate R, Neri Serneri GG (1997) Tissue factor and plasminogen activator inhibitor type 2 expression in human stimulated monocytes is inhibited by heparin. Semin Thromb Hemost 23:135–141
30. Marsh NA, Minter AJ, Chesterman CN (1990) The effect of heparin and other glycosaminoglycans on levels of tissue plasminogen activator and plasminogen activator inhibitor in cultured human umbilical vein endothelial cells. Blood Coagul Fibrinolysis 1:133–138
31. Nuckton TJ, Alonso JA, Kallet RH, Daniel BM, Pittet JF, Eisner MD, Matthay MA (2002) Pulmonary dead-space fraction as a risk factor for death in the acute respiratory distress syndrome. N Engl J Med 346:1281–1286
32. Askrog V, Pender J, Eckenhoff J (1964) Changes in the physiological dead space during deliberate hypotension. Anesthesiology 25:744–751
33. Nunn JF (1977) Respiratory dead space. In: Applied respiratory physiology, 2nd edn. Butterworths, London
34. Chandler WL, Velan T (2003) Estimating the rate of thrombin and fibrin generation in vivo during cardiopulmonary bypass. Blood 101:4355–4362