BJA

British Journal of Anaesthesia, 116 (2): 223–32 (2016)

doi: 10.1093/bja/aev411
Cardiovascular

Endothelial hyperpermeability after cardiac surgery with cardiopulmonary bypass as assessed using an *in vitro* bioassay for endothelial barrier function

N. J. Koning[1], M. A. H. Overmars[1], C. E. van den Brom[1], J. van Bezu[2], L. E. Simon[1], A. B. A. Vonk[3], A. R. J. Girbes[4], G. P. van Nieuw Amerongen[2] and C. Boer[1,*]

[1]Department of Anesthesiology, [2]Laboratory for Physiology, Institute for Cardiovascular Research, [3]Department of Cardio-thoracic Surgery, and [4]Department of Intensive Care Medicine, VU University Medical Centre, De Boelelaan 1117, Amsterdam 1081 HV, The Netherlands

*Corresponding author: E-mail: c.boer@vumc.nl

Abstract

Background: The mechanisms causing increased endothelial permeability after cardiopulmonary bypass (CPB) have not been elucidated. Using a bioassay for endothelial barrier function, we investigated whether endothelial hyperpermeability is associated with alterations in plasma endothelial activation and adhesion markers and can be attenuated by the use of pulsatile flow during CPB.

Methods: Patients undergoing cardiac surgery were randomized to non-pulsatile (n=20) or pulsatile flow CPB (n=20). Plasma samples were obtained before (pre-CPB) and after CPB (post-CPB), and upon intensive care unit (ICU) arrival. Changes in plasma endothelial activation and adhesion markers were determined by enzyme-linked immunosorbent assay. Using electric cell–substrate impedance sensing of human umbilical vein endothelial monolayers, the effects of plasma exposure on endothelial barrier function were assessed and expressed as resistance.

Results: Cardiopulmonary bypass was associated with increased P-selectin, vascular cell adhesion molecule-1, and von Willebrand factor plasma concentrations and an increase in the angiopoietin-2 to angiopoietin-1 ratio, irrespective of the flow profile. Plasma samples obtained after CPB induced loss of endothelial resistance of 21 and 23% in non-pulsatile and pulsatile flow groups, respectively. The negative effect on endothelial cell barrier function was still present with exposure to plasma obtained upon ICU admission. The reduction in endothelial resistance after exposure to post-CPB plasma could not be explained by CPB-induced haemodilution.

Conclusions: The change in the plasma fingerprint during CPB is associated with impairment of *in vitro* endothelial barrier function, which occurs irrespective of the application of a protective pulsatile flow profile during CPB.

Clinical trial registration: NTR2940.

Key words: capillary permeability; cardiopulmonary bypass; endothelium, vascular; haemodilution; pulsatile flow

Patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) are at risk of developing tissue oedema as a result of inflammatory processes, fluid overload, and augmented fluid extravasation.[1 2] Postoperative oedema may contribute to the development of complications, in particular acute lung injury.[3]

Accepted: September 13, 2015

Editor's key points

- The mechanisms by which cardiopulmonary bypass (CPB) increases endothelial permeability are not known.
- Possible mechanisms include reductions in shear stress associated with non-pulsatile flow, and inflammatory responses.
- The authors exposed endothelial cell layers to blood obtained from patients undergoing CPB.
- Impaired endothelial function was associated with increased plasma concentrations of endothelial activation and adhesion proteins.

Fluid extravasation during cardiac surgery is mainly a result of altered gradients in the hydrostatic and oncotic pressure attributable to crystalloid and colloid fluid administration, loss of glycocalyx and basement membrane integrity, and endothelial barrier dysfunction.[4–6] The aetiology of endothelial hyperpermeability during cardiac surgery is, however, not well understood. Previous studies suggested that endothelial barrier function during CPB is attenuated by volatile anaesthesia,[7] a non-pulsatile blood flow profile,[8 9] the release of pro-inflammatory mediators,[10 11] endothelial activation,[11–13] haemodilution,[14] the use of heparin,[15] and hypothermia.[16] In studies in pigs, CPB was associated with degradation of endothelial adherens junctions in tissue sections,[17] reduced myocardial vascular VE-cadherin, β-catenin, and γ-catenin concentrations,[18] and fluid extravasation.[7 19 20] This could not be diminished with anti-inflammatory agents[16] or pulsatile blood flow.[19]

Most of the aforementioned findings were obtained in animal studies[7 14 16–20] or limited to the evaluation of plasma markers[8 10 11] and immunohistochemistry in arterial biopsies.[12] Moreover, the data are inconclusive regarding whether loss of endothelial barrier function during CPB is dependent on the direct effect of non-pulsatile blood flow associated with reduced endothelial shear stress[21 22] or specifically mediated by alterations in the plasma footprint of biomarkers. We have previously performed a randomized controlled trial of the influence of pulsatile CPB on microcirculatory perfusion.[23] *A priori*-defined secondary outcomes of that study were endothelial biomarker expression and barrier function. In the present study we therefore used blood from our previous study and a bioassay for endothelial barrier function to investigate the effect of cardiac surgery with non-pulsatile or pulsatile CPB on endothelial cell permeability.[23]

Methods

Study design

The study was approved by the Human Subjects Committee of the VU University Medical Centre (NL34947.029.10) and pre-registered on the Dutch Trial Registry (NTR2940). All participants provided written informed consent to participate in this single-centre randomized controlled trial. Patients aged 50–85 yr undergoing elective coronary artery bypass graft surgery with CPB were included. Exclusion criteria were an emergency operation or reoperation, insulin-dependent diabetes mellitus, or anaemia (haemoglobin <8.9 mg dl^{-1}). Patients were assigned to the non-pulsatile flow or pulsatile flow group by randomization using envelope drawing. The study flow diagram is shown as Supplementary File S1.

Anaesthesia and surgery

A previously described standard anaesthesia protocol was used in all patients.[23] Patients received dexamethasone (1 mg kg^{-1}), cefazolin (1000 mg) and tranexamic acid (2 g) according to local protocols. Heparin (300 IU kg^{-1}) was used to achieve an activated clotting time of >480 s. The extracorporeal circuit consisted of a centrifugal pump head (Sorin, Mirandola, Italy), a polyvinyl heparin-coated tubing system, and a hollow fibre oxygenator (Affinity; Medtronic, Minneapolis, MN, USA). The system was primed with 1 litre of Gelofusin® (Braun, Melsungen, Germany), 250 ml lactated Ringer's solution (Baxter BV, Utrecht, Netherlands) containing cefalozin (1000 mg; Eli Lilly Nederland BV, Nieuwegein, Netherlands), mannitol (100 ml of 20%; Baxter BV), sodium bicarbonate (50 ml of 8.4%; Braun Melsunger AG), and porcine heparin (5000 IU). Cardiopulmonary bypass blood flow was maintained at 2.2–3.0 litre min^{-1} m^{-2} with mild hypothermia (34°C). Heparin was neutralized by protamine. A cell saver (Autolog; Medtronic) was routinely used for autologous red blood cell concentration. During aortic cross-clamping, the flow character of the centrifugal pump was set to non-pulsatile in the non-pulsatile flow group or pulsatile in the pulsatile flow group (60 accelerations min^{-1}) according to a standard CPB protocol.[23] Analysis of the area under the curve (AUC) was performed on the arterial blood pressure waveform before CPB and during aortic cross-clamping in order to quantify the pulsatility delivered by the centrifugal pump.

Blood sampling

Blood samples were collected before induction of anaesthesia (pre-CPB) and 3 min (post-CPB) and 60 min [in the intensive care unit (ICU)] after protamine infusion, and immediately centrifuged at 2500 G during 10 min at 4°C. The plasma supernatant was centrifuged for 5 min at 13500 G to achieve platelet-free plasma. The platelet-free plasma was snap frozen in liquid nitrogen and stored at −80°C.

Plasma analysis

Plasma samples were analysed for P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), von Willebrand factor (vWF), vascular endothelial growth factor (VEGF; all from AbCam, Cambridge, UK), angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), and soluble Tie2 (sTie2; R&D Systems, Minneapolis, MN, USA) concentrations using commercially available enzyme-linked immunosorbent assays. Additionally, post-CPB and ICU values were corrected for dilution attributable to CPB as reflected by the relative decrease in haematocrit immediately after onset of CPB compared with pre-CPB haematocrit values.

Human umbilical vein endothelial cells

Human umbilical vein endothelial cells (HUVECs) were isolated and cultured as described before.[24] The HUVECs were isolated from human umbilical cords and subsequently cultured on gelatine-coated well plates in M199 medium at 37°C, in an atmosphere of 95% air and 5% CO_2.

Electric cell–substrate impedance sensing

Electric cell–substrate impedance sensing (ECIS) is a technique to measure impedance of endothelial cells (Applied BioPhysics, Troy, NY, USA), as previously described.[24 25] Using a 96-well ECIS array (0.3 cm^2 per well containing 20 gold electrodes of 350