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Blood transfusions may impair endothelium-dependent vasodilatation during coronary artery bypass surgery☆

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ABSTRACT

Objective: The hemolytic product free-hemoglobin (fHb) reduces nitric oxide (NO) bioavailability. The present study aims to establish whether administration of different blood transfusions result in increased circulating fHb levels and NO consumption with effects on arterial NO-dependent blood flow in patients undergoing CABG surgery.
Methods: Ninety-five consecutive patients undergoing elective CABG surgery were prospectively divided in four groups based on blood transfusion requirements during surgery: stored blood cells (SBC, n. 21), intraoperative autologous salvaged blood (ASB, n. 25), SBC and ASB (n.22), no transfusion (control, n. 27).
Blood samples were collected before and after intervention to analyse plasma levels of fHb and NO consumption. Endothelium-dependent relaxation was assessed in left internal mammary artery (LIMA) rings harvested before chest closure. Peripheral artery tonometry was assessed after intervention.
Results: Transfusions with SBC increased plasma fHb ($p < 0.05$). Transfusions of ASB resulted in higher plasma fHb compared to SBC ($p < 0.01$). fHb concentrations directly correlated with NO consumption ($r = 0.65$, $p < 0.001$). Maximal endothelium-dependent relaxation in LIMA was significantly attenuated in SBC and ASB patients compared to control (15.2 ± 3.1% vs 21.1 ± 2.5% vs 43 ± 5.0% respectively; $p < 0.01$). Significant correlations were identified between the aortic pressure wave velocity, plasma fHb concentration and NO consumption ($p < 0.01$).
Conclusions: Intraoperative blood transfusions and particularly autologous salvaged blood impair endothelium-dependent relaxation through NO scavenging by fHb. These findings obtained in vitro and in vivo provide new insights into the adverse relation between blood transfusions and patient outcome.

1. Introduction

Several studies have implicated red blood cell (RBC) transfusion as a risk factor for increased morbidity as well as short- and long-term mortality after coronary artery bypass graft surgery (CABG) (Engoren et al., 2002; Koch et al., 2006). Interestingly, this effect is most pronounced among patients undergoing isolated CABG, suggesting that a harmful mechanism of RBC transfusion is not generalizable to all types of surgical procedures but instead may be specific to those patients (Engoren et al., 2015).

The mechanisms by which blood transfusion may contribute to organ injury, dysfunction and death remain uncertain. Besides the quantity of RBC transfusions (Paone et al., 2014), the quality of the product has also been associated with differences in mortality and morbidity (Voorhuis et al., 2013).

The use of intraoperative cell salvage and autologous blood transfusion has become an important method of blood conservation. The main aim of autologous transfusion is to reduce the need for allogeneic blood transfusion and its associated complications. However hemolysis and high cell-free hemoglobin (fHb) contents are associated with cell washing devices that collect anticoagulated shed or recovered blood, wash and separate the RBC by centrifugation, and re-infuse the RBC (Niranjan et al., 2006).

fHb is a potent scavenger of nitric oxide (NO), which mediates endothelium-dependent vasodilation, and may therefore be responsible for microvascular perfusion disturbances (arterial spasm) (Rungatscher et

Abbreviations: fHb, free hemoglobin; NO, nitric oxide; SBC, stored blood cells; ASB, autologous salvaged blood cells; LIMA, left internal mammary artery; RBC, red blood cells; PWV, pressure wave velocity; AI, augmentation index.

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al., 2010). Indeed endothelial dysfunction and persistent microvascular alterations are associated with organ failure and death (Coletta et al., 2014).

Increasing hemolysis and release of fHb were also documented as a function of time during prolonged RBC storage (Donadee et al., 2011). Transfusion of RBC stored for <14 days has been associated with favourable outcomes when compared with transfusion of RBC stored for a prolonged time (Koch et al., 2008; Moskowitz et al., 2010). Changes in red cell structure and function during blood banking and storage have been referred to as the red cell storage lesion (Card et al., 1982; Kim-Shapiro et al., 2011).

The present study aimed to assess whether transfusion of stored allogeneic blood or intraoperative autologous salvaged blood results in differently increased circulating fHb levels and plasma NO consumption with effects on arterial NO-dependent blood flow in patients undergoing CABG surgery.

2. Methods

2.1. Study design

This single-centre, prospective observational cohort study was based on consecutive patients undergoing elective isolated CABG with cardiopulmonary bypass (CPB) at our institution between January 2014 and May 2014. Perioperative and postoperative data were collected prospectively. The study protocol was approved by the Institution's Ethical Committee/Institutional Review Board and the trial was registered: Clinicaltrials.gov NCT02953951

2.2. Surgery

Anesthesia was standardized and maintained with propofol, sufentanil and vecuronium. Surgery was always performed through median sternotomy. LIMA was harvested as a pedicle, anastomosed to the left anterior descending artery in all cases, and never used as a free graft. CPB circuit included a Sorin phosphorylcholine-coated tubing set (Sorin Group SpA, Milano, Italy), a Jostra roller pump (Jostra, Maquet Cardiopulmonary, Hirrlingen, Germany), and a hollow fiber membrane coated oxygenator, which incorporated also a 40 μm filter (Sorin Synthesis™, Sorin Group Spa, Milano, Italy). Heparin was given at a dose of 300 IU/kg to achieve a target activated clotting time of 480 s or above. The extracorporeal circuit was primed with 1400 mL of Ringer's lactate solution and 5000 IU of heparin. A non-pulsatile CPB flow was established at 2.4 L/min. Buckberg crystalloid cardioplegic solution was used to maintain cardioplegia during aortic cross-clamping. Infusion of 4 mg/kg of protamine was used to neutralize heparin after finishing the CPB. Residual blood was sucked from the venous reservoir into the cell saver collection reservoir using a dual lumen tube connected to a vacuum pump and subsequently transferred to the 125-mL centrifuge bowl of the cell saver (Cell Saver 5; Haemonetics, Braintree, MA, USA). Cells were washed using 2000 mL bags NaCl 0.9% with 30.000 IU of heparin (Athena Pharma, Rome, Italy). The following cell saver program was used for the cell saver: 5600 r.p.m., filling and washing rate of 250 mL/min, emptying rate 250 mL/min, wash volume 1000 mL. Concentrated blood cells were drained into a patient labelled soft collection bag.

2.3. Transfusion protocol

Transfusion of packed RBC was indicated at Hb levels <7 g/L during CPB. After the end of CPB an Hb level < 9 g/L indicated requirement of autologous salvaged concentrated blood cells and/or packed RBC.

2.4. Group assignment

For data analysis, patients were divided into four group: group 1 consisted of patients who received packed stored blood cells (designated "SBC" group); group 2: patients who received autologous salvaged blood (designated "ASB" group); group 3: patients who received both stored blood cells and autologous salvaged blood (designated "SBC + ASB" group); group 4: patients who did not received blood transfusion ("control" group).

Patients who received vasopressors/inotropes during surgery were excluded from analysis.

2.5. Measurements

2.5.1. Free hemoglobin

The fHb concentrations were measured in all patient plasma samples before anesthesia (baseline), during CPB (before transfusion) and at intensive care unit arrival (after transfusion). Moreover fHB was assessed in the storage medium of every administered SBC and ASB. fHb was analysed by derivative spectrometry (Cruz-Landeira et al., 2002). The detection limit of this assay was 2 μmol/L.

2.5.2. Nitric oxide consumption assay

The NO consumption was measured in all patient plasma samples obtained at the same time of those collected for fHb. The complete protocol of the NO consumption assay was already described (Wang et al., 2004). In short, a 40 μM solution of the NO donor (DETA NONOate; Cayman Chemical, Ann Harbor, MI, USA) was prepared in PBS (pH 7.4) in a glass vessel purged with nitrogen in line with a NO chemiluminescence analyzer (Sievers Model 280i; GE, Boulder, CO, USA). The subsequent decay of DETA NONOate, releasing NO, produced a steady state NO signal of about 50 to 70 mV. When the signal became stable, 50 μL plasma samples or standards were injected into the DETA NONOate solution. In cases of NO consumption the signal decreases. Data were transferred to the software program ORIGIN Version 6.1 (OriginLab, Northampton, MA, USA) for analysis of the area under the curve of the decreasing NO signal over time.

2.5.3. Endothelium-dependent relaxation in isolated left mammary artery rings

At the end of surgery, discarded LIMA fragments were placed in cold (4 °C) calcium free modified Krebs-Henseleit solution of the following composition: NaCl, 123.0; KCl, 4.70; $MgSO_4$, 1.64; $NaHCO_3$, 24.88; KH_2PO_4, 1.18; glucose, 5.55; sodium pyruvate, 2.0 (mM) (pH 7.4). Upon arrival at laboratory the vessels were divided into 3 mm long segments. The arterial rings were suspended on stainless steel wire hooks in the organ bath chamber (20 mL) filled with oxygenated (95% O_2, 5% CO_2) Krebs Henseleit solution of the following composition: NaCl, 119.0; KCl, 4.70; CaCl2, 1.6; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; glucose, 11.01; sodium pyruvate 2.0 (mM) (pH 7.4) and in presence of plasma (10 mL) collected at the same time and from the same patient from which the LIMA was harvested. The temperature was maintained at 37 °C and the arterial rings were equilibrated for 30 min. The Schuler isolated organ bath (HSE; March-Hugstetten, Germany) was used. Vessel wall tension was measured with isometric force transducer F 30 (HSE), the signal was enhanced with bridge amplifier Type 336 (HSE) and recorded using PowerLab/4SP system and Chart software (AD Instruments, Chalgrove, Oxfordshire, UK). Next a maximal preconstriction was induced exposing the rings to Krebs buffer that contained KCl at final concentration of 100 mmol/L. After this, the rings were carefully rinsed several times and preconstricted to 60% to the maximal KCl-induced tone by adding increasing dosages of phenylephrine (Sigma, Milan, Italy; 10^{-9} to 3×10^{-4} mol/L). Endothelium-dependent relaxation in response to acetylcholine (Sigma; 10^{-11} to 10^{-5} mol/L was recorded.