Mini Bypass and Proinflammatory Leukocyte Activation: A Randomized Controlled Trial

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

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

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

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

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Coronary artery bypass grafting (CABG) is frequently performed with cardiopulmonary bypass (CPB). However, CPB is known to evoke a systemic inflammatory response [1]. Miniaturized CPB has been developed to attenuate systemic inflammation by optimizing the components of the CPB circuit. Optimization may be achieved through reducing hemodilution by minimizing 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 (eg, phosphorylcholine), which would be expected to reduce contact activation. In previous studies, mCPB was 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 operations using CPB induces systemic inflammation, the proinflammatory signaling pathways activated by CPB have not been precisely defined. Moreover, the cell-signaling mechanisms underlying the inflammatory effects of CPB miniaturization have not been characterized at a molecular level. To address this issue, a clinical trial to determine the kinetics of inflammatory signaling pathway activation was performed in patients undergoing CABG with conventional CPB (cCPB) or miniaturized CPB (mCPB). The primary hypothesis was that compared with cCPB, the mCPB system would reduce reactive oxygen species (ROS)/ proinflammatory 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.

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Material and Methods

Study Design

A single-center, parallel-group randomized controlled trial was performed (ISRCTN30610605). A favorable 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 performed by a single surgeon were considered. Patients younger than 18 years of age, emergency operations, ejection fraction less than 30%, recent cerebrovascular accident, greater than 75% carotid artery stenosis, renal impairment (serum creatinine level >177 μmol/L), preexisting coagulopathy, preexisting liver dysfunction, or recent (within 5 days) use of antiplatelet agents (aspirin/clopidogrel) were reasons for exclusion.

Intervention and Comparator

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

Operations using cCPB or mCPB involved aortic cannulation, 2-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 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, because these were considered the most rapid markers of proinflammatory signaling within the cell lineage first activated in acute inflammation [12]. Secondary outcomes were ROS activation in monocytes and lymphocytes; activation of proinflammatory p38 mitogen-activated protein kinase (MAPK), which promotes inflammation by activating downstream AP-1 superfamily transcription factors [13], and activation of nuclear factor (NF)-κB transcription factor, which activates multiple proinflammatory genes [14], measured as the phosphorylated p65 subunit. Secondary outcomes also included leukocyte counts in cantharidin-induced blisters, white cell counts, and serum C-reactive protein (CRP) and creatinine levels. Sampling times are summarized 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 (Molecular Probes, Eugene, OR) for 30 minutes at 37°C before erythrocyte lysis. In parallel, leukocytes were fixed with phosphatase buffers and isolated using erythrocyte lysis and centrifugation using manufacturer-published BD PhosFlow (BD Biosciences, San Jose, CA) methods before incubation with phycoerythrin (PE)-Cy7–conjugated antibodies that recognize dual Thr180/Tyr182–phosphorylated p38-MAPK and PE-conjugated antibodies that recognize Ser529-phosphorylated NF-κB (BD Biosciences, San Jose, CA). Fluorescence was quantified in granulocytes, monocytes, and lymphocytes (forward and side scatter) using flow cytometry and Summit 4.3 software (Beckman-Coulter, Brea, CA).

Quantitation of Leukocyte Migration

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

Sample Size

Although the primary outcome was intracellular ROS activation in leukocytes, the target sample size was

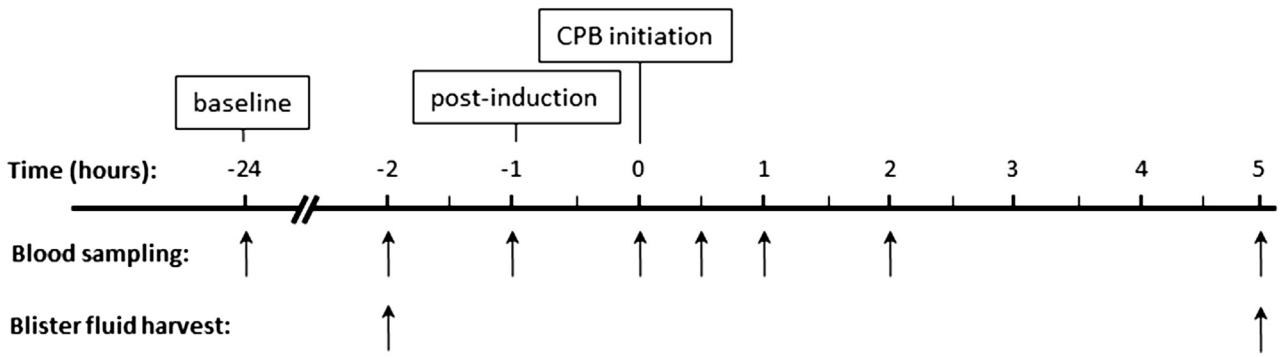

Fig 1. Summary of blood and blister fluid sampling times. Blood and cantharidin-induced skin blister fluid were sampled in patients exposed to conventional cardiopulmonary bypass (cCPB) and those who underwent miniaturized cardiopulmonary bypass (mCPB). A timeline is presented to summarize sampling times.