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ORIGINAL ARTICLE

Mitochondrial DAMPs Are Released During Cardiopulmonary Bypass Surgery and Are Associated With Postoperative Atrial Fibrillation

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Q5 **Background** Atrial fibrillation (AF) is the most frequent complication of surgery performed on cardiopulmonary bypass (CPB) and recent work associates CPB with postoperative inflammation. We have shown that all tissue injury releases mitochondrial damage associated molecular patterns (mtDAMPs) including mitochondrial DNA (mtDNA). This can act as a direct, early activator of neutrophils (PMN), eliciting the systemic inflammatory response syndrome (SIRS) while suppressing PMN function. Neutrophil Extracellular Traps (NETs) are crucial to host defence and carry out NETosis whereby webs of granule proteins and chromatin trap and kill bacteria. We hypothesised that surgery performed on CPB releases mtDAMPs into the Q6 circulation. Molecular patterns thus mobilised during CPB might then participate in the pathogenesis of inflammatory postoperative complications and be a predictor of impending complications [1].

Methods We prospectively studied 16 patients undergoing elective operations on CPB. Blood was sampled preoperatively, at the end of CPB and days 1–2 postoperatively. Plasma samples were analysed for mtDNA. Neutrophil IL-6 gene expression was studied to assess induction of SIRS. Neutrophils were also assayed for the presence of neutrophil extracellular traps (NETs/NETosis). The biologic findings were then correlated to clinical data and compared in patients with and without postoperative AF (POAF).

Results Mitochondrial DNA was significantly elevated following CPB (six-fold increase post-CPB, $p = 0.008$ and five-fold increase days 1–2, $p = 0.02$). Patients with POAF showed greater increases in mtDNA post-CPB than those without. Postoperative AF was seen in all patients with a $\geq$2-fold increase of mtDNA ($p = 0.037$ vs. $<$2-fold). Neutrophil IL-6 gene regulation increased postoperatively demonstrating SIRS that was greatest days 1–2 ($p = 0.039$). Neutrophil extracellular trap (NET) formation was markedly suppressed in the post-CPB state.

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Conclusion Mitochondrial DNA is released by CPB surgery and is associated with POAF. IL-6 gene expression increases after CPB, demonstrating the evolution of postoperative SIRS. Lastly, cardiac surgery on CPB also suppressed PMN NETosis. Taken together, our data suggest that mtDNA released during surgery on CPB, may be involved in the pathogenesis of SIRS and related postoperative inflammatory events like POAF and infections. Mitochondrial DNA may therefore prove to be an early biomarker for postoperative complications with the degree of association to be determined in appropriately sized studies. If mtDNA is directly involved in cardiac inflammation, mtDNA-induced toll-like receptor-9 (TLR9) signalling could also be targeted therapeutically.

Keywords Mitochondrial DAMPS • Damage molecules • Cardiopulmonary bypass • Atrial fibrillation • Inflammation

Introduction

Q7 Postoperative atrial fibrillation (POAF) is a major clinical problem after cardiac operations occurring in 30–50% of patients [2], and is thus the most common postoperative arrhythmia [2,3]. Atrial fibrillation can result in haemodynamic compromise and is associated with an increased risk of thromboembolic events like stroke. Finally, POAF can also be associated with increased bleeding events in those patients who require anticoagulant therapy [2]. Thus patients who develop POAF can also require increased hospital length of stay [2], predisposing to a higher risk of other postoperative complications such as stroke and perioperative myocardial infarction [4]. Postoperative AF can therefore contribute significantly to overall outcomes following cardiac surgery. Q8

Many of the demographic factors leading to cardiac surgery are themselves risk factors for POAF, but within this population there are no reliable predictive tests for AF. Moreover, no unifying mechanism has been proposed that explains the connection between cardiac surgery and POAF. Recently however, there has been increased interest in the link between POAF and inflammation. Interleukin-6 (IL-6) and C-reactive protein (CRP) levels measured in the immediate postoperative period have been shown to be independent predictors of POAF [5–7]. Thus, several studies have addressed the use of anti-inflammatory agents postoperatively: colchicine administered after pulmonary vein isolation significantly reduced levels of IL-6 and CRP postoperatively and decreased AF recurrence [8]. Postoperative AF was also decreased in post-pericardectomy patients given colchicine [9,10].

Cardiopulmonary bypass appears to be a potent initiator of systemic inflammatory response syndrome (SIRS) although mechanistic links between SIRS and CPB are not well defined. Studies so far report contact activation, ischaemia-reperfusion injury, complement cascade and endotoxaemia as plausible causes [11], but there is no unified understanding to date. We previously showed that tissue injury preceded by trauma and cell necrosis, releases mtDAMPs [12] including mtDNA, that activate circulating leukocytes and could activate cardiomyocytes through interactions with TLR9 [13,14]. In neutrophils (PMN) this is associated with clinical initiation of SIRS [12] but the role of mtDNA-TLR9 in clinical activation of cardiomyocytes by SIRS has never been studied even though digoxin, which is used clinically to treat AF, has been shown to suppress myocardial inflammation [15,16].

Finally, SIRS after trauma has been shown to be linked to diminished PMN function and our prior work suggests that mtDAMPs release is associated with suppression of neutrophil extracellular traps (NETs) [17], which are required for PMN trapping and killing of bacteria [18,19]. We therefore hypothesised that mtDNA released during surgery on CPB could be related to the occurrence of POAF and that CPB might also affect PMN function by NETosis.

Materials and Methods

Patient Selection and Blood Sample Collection

This study was approved by the Institutional Research Board of Beth Israel Deaconess Medical Center. Written consent was obtained from all patients. Blood samples were collected either through existing arterial or central venous catheters or during a scheduled postoperative phlebotomy. Adult patients undergoing open cardiac operations on CPB were all considered for study without any specific exclusion criteria if they could provide consent.

Blood Sample Collection

Blood samples (12–18 mL) were obtained in tubes containing Ethylenediaminetetraacetic acid (EDTA). Samples were collected 1) preoperatively, 2) immediately after CPB (within 90 minutes of decannulation) and 3) on days 1–2 postoperatively.

Reagents and Chemicals

Phosphate-buffered saline was purchased from Sigma Aldrich (St Louis, USA), and RPMI medium was purchased from GIBCO (Invitrogen, Grand Island, NY). All other materials were obtained as outlined below.

DNA Isolation From Plasma

Whole blood was centrifuged at 200 × g for 10 minutes at room temperature to obtain plasma and cells for subsequent PMN separation (see below). The plasma thus obtained was spun a second time at 5000 × g, at 4 °C for 10 minutes to

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