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Angiopoietin-2 is a potential mediator of endothelial barrier dysfunction following cardiopulmonary bypass

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ABSTRACT

Introduction: Endothelial activation leading to vascular barrier dysfunction and organ failure is a well-recognized complication of cardiovascular surgery with cardiopulmonary bypass (CPB). The endothelial-specific angiopoietin–Tie2 ligand–receptor system has been identified as a non-redundant regulator of endothelial activation. Binding of angiopoietin-2 (Ang-2) to the Tie2 receptor antagonizes Tie2 signaling and renders the endothelial barrier responsive to pro-inflammatory cytokines. We aimed to study the time course and potential triggering factors of Ang-2 release after CPB, as well as the association of Ang-2 changes with surrogates of increased vascular permeability, organ dysfunction, and outcome.
Methods: Serum levels of Ang-2 from 25 adult patients (140 screened) were measured before and at 0, 12, and 24 h following CPB procedure by in-house immuno-luminometric assay (ILMA), and compared with indices of organ dysfunction, duration of mechanical ventilation (MV), length of stay (LOS) in the intensive care unit (ICU), and hospital mortality. The effect of Ang-2 was studied *in vitro* by incubating high Ang-2 patient serum with endothelial cells (EC).
Results: Ang-2 levels steadily increased from 2.6 ± 2.4 ng/mL at 0 h up to 7.3 ± 4.6 ng/mL at 24 h following CPB ($P < 0.001$). The release of Ang-2 correlated with the duration of CPB, aortic cross-clamp time, and post-CPB lactate levels. Changes in Ang-2 during follow-up correlated with partial pressure of oxygen in arterial blood (PaO_2)/fraction of inspired oxygen (FiO_2) ratio, alveolar–arterial oxygen tension difference ($AaDO_2$), hemodynamics, fluid balance, and disease severity measures. Ang-2 levels at 12 h predicted the duration of MV, ICU-LOS, and hospital mortality. High Ang-2 patient sera disrupted EC architecture *in vitro*, an effect reversed by treatment with the competitive Tie2 ligand angiopoietin-1 (Ang-1).
Conclusions: Collectively, our results suggest that Ang-2 is a putative mediator of endothelial barrier dysfunction after CPB. These findings suggest that targeting the Ang/Tie2 pathway may mitigate organ dysfunction and improve outcome in patients undergoing CPB.

1. Introduction

The vascular endothelium constitutes a key player in the pathogenesis of organ dysfunction [1]. It is particularly sensitive to the side effects of cardiopulmonary bypass (CPB), which include complement and platelet activation as well as consumption, and the release of a multitude of proinflammatory cytokines [2,3]. As a result, the phenotype of the vascular endothelium changes from a quiescent, anticoagulant state to an activated, procoagulant state, which is paralleled by disassembly of adherence junctions, myosin driven cell contraction and subsequent inter-endothelial gap formation [4].

Abbreviations: CPB, cardiopulmonary bypass; Ang-2, angiopoietin-2; ILMA, immuno-luminometric assay; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment; LOS, length of stay; ICU, intensive care unit; EC, endothelial cells; AKI, acute kidney injury; ALI, acute lung injury; Ang-1, angiopoietin-1; MV, mechanical ventilation; SD, standard deviation; ASF, actin stress fibers.

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This highly regulated cascade of events lead to net extravasation of fluid, which contributes to hypovolemia, tissue edema, and eventually organ dysfunction. Consistently, devastating organ injuries such as acute kidney injury (AKI) or acute lung injury (ALI) are among the most frequent complications after on-pump cardiac surgery [5–8].

Angiopoietins are angiogenic factors essential for vascular development, maturation, and inflammation [9–12]. As circulating or matrix-bound molecules, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) bind to the extracellular domain of the tyrosine kinase receptor Tie2, predominantly expressed on endothelial cells [13,14]. Operational Ang-1/Tie2 signaling prevents endothelial cells apoptosis by activating the phosphoinositol 3-kinase/Akt survival pathway [34,36]. Moreover, Ang-t1 decreases vascular permeability through coordinated and opposite effects on the Rho GTPases Rac1 and RhoA which in turn restrict the number and size of gaps that form at endothelial cell junctions in response to various leakage-inducing agents such as bradykinine, thrombin, VEGF, or TNF-α [12,15,16]. Constitutive Ang-1 expression by vascular mural cells, and low-level Tie2 phosphorylation, probably represent a non-redundant control pathway that maintains vessel integrity, prevents endothelial hyperpermeability and inhibits leukocyte–endothelium interactions [9,17]. Upon a variety of stimuli the endogenous context-specific Tie-2 antagonist, Ang-2, is rapidly released by the activated endothelium from so-called Weibel-Palade bodies [18] and disrupts constitutive Ang-1/Tie2 signaling by preventing Ang-1 from binding to the receptor [13,18,19]. Cellular experiments have shown that Ang-2-mediated endothelial destabilization results from complex formation between Tie2 and αvβ3 with subsequent integrin internalization and degradation [20].

We and others have shown that Ang-2 levels in plasma from critically-ill septic patients correlate with the extent of pulmonary vascular leak and acute lung injury [21,22], increase with the severity of multiple-organ dysfunction syndrome [23,24], and independently predict mortality in the intensive care unit (ICU) [23–28]. Recently, Giuliano et al. were the first to show that plasma Ang-2 levels increased early after CPB in children [29]. We therefore hypothesized that Ang-2 release following CPB correlates with surrogates of capillary leakage and outcome in adults. In addition, we performed *in vitro* experiments with patients' serum before and after CPB to test the contributory role of Ang-2 on endothelial dysfunction.

2. Methods

2.1. Study design and patient population

From May to June 2009 we screened 140 patients scheduled for elective heart surgery with CPB at Hannover Medical School. To ensure a well-defined and homogenous study population several inclusion and exclusion criteria were defined as follows. Inclusion criteria were age $\geqslant$ 18 years, scheduled major cardiac on-pump surgery, and willingness to provide written informed consent. Exclusion criteria included conditions that are associated with elevated Ang-2 levels *per se*, such as emergency surgery, severe congestive heart failure (ejection fraction < 20%), active malignancy [30], previous organ transplantation or any disease that requires immunosuppressive drugs during the past 6 months, active or uncontrolled viral infection (Hepatitis/HIV), active acute infectious disease and/or severe chronic infectious disease requiring antibiotic treatment [23,31], advanced chronic kidney disease (estimated GFR [MDRD formula] < 30 mL/min or requiring any kind of dialysis) [27] and pregnancy [32].

Enrollment was performed in a consecutive fashion after obtaining written informed consent from the patients or their legal representatives. The study was performed in accordance with the declaration of Helsinki and approved by the institutional review board.

2.2. Evaluation

Patient demographics, including age at surgery, weight, diagnosis, duration of CPB, duration of aortic cross-clamp, peri-operative fluid balance at 24 h following CPB (the total fluids in − the total fluids out), duration of mechanical ventilation (MV), ICU-LOS, and dose of inotropic substances were prospectively collected. Routine laboratory data were determined twice daily after surgery. Sequential Organ Failure Assessment (SOFA) score [33] and Simplified Acute Physiology Score (SAPS II) score [34] were calculated at 0, 24, and 48 h after ICU admission. For spontaneously breathing patients in whom the arterial line was removed, the PaO_2/FiO_2 ratio was set >300 mmHg to complete the score calculations at 48 h after CPB.

2.3. Intraoperative management

Operations were performed according to in house standard operating procedures. In brief, sodium thiopental, fentanyl and pancuronium bromide were administered to all patients. All patients underwent routine median sternotomy, in coronary artery bypass graft patients the left internal mammary was prepared. Prior to CPB, patients received heparin (300 U/kg) and an activated clotting time of more than 400 s was maintained thereafter. CPB was established *via* cannulation of the ascending aorta and right atrium using a heparin-coated circuit, a roller pump (Stockert Instrumentation, Munich, Germany) and a membrane oxygenator (Monolyth; Sorin Biomedica, Munich, Germany). In case of mitral valve surgery, double venous cannulation was established in the superior and inferior vena cava. During CPB a mean arterial pressure of 50 to 70 mmHg and moderate hypothermia (30–32 °C) was maintained. For cardioplegia, St. Thomas' solution (1–1.5 L) was infused through the aortic root or direct ostial cannulation to achieve myocardial preservation during cross-clamping. At the completion of surgery, patients were warmed to a minimum of 36.5 °C before CPB was weaned off, after which heparin was reversed completely. Upon completion of the operation, all patients were immediately transported to the surgical intensive care unit for recovery.

2.4. Sampling and quantification of circulating angiopoietin-2 and endothelial adhesion molecules

Baseline blood samples were collected within 24 h before the surgical procedure. Additional blood samples were obtained at 0, 12 and 24 h after the surgical procedure. Immediately after procurement, blood samples were centrifuged at 2.000 G for 10 min, divided into aliquots and stored at −80 °C. Routine chemistry test and blood gas analyses were performed in parallel. Serum Ang-2 levels were measured by in-house immuno-luminometric assay (ILMA) using human Ang-2 monoclonal Ang-2-antibody and anti-Ang-2-antibody Ang-2 monoclonal methodology (R&D, Oxon, UK) as described previously [24,30]. The assay had a detection limit of 0.2 ng/mL. Inter-assay and intra-assay imprecision is $\leqslant$ 4.6% and 5.2%, respectively. All measurements were performed in duplicates at the same day by the same investigator blinded to patients' characteristics and outcome. Mean serum Ang-2 levels in 29 apparently healthy subjects (59 ± 18 years of age) were 1.0 ± 0.5 ng/mL. Serum levels of soluble vascular-cell adhesion molecule-1 (sVCAM-1) and soluble E-selectin were quantified by bead-based flow cytometry assay (FlowCytomix, eBioscience, Frankfurt, Germany) according to the manufacturer's instructions.