Increased vascular permeability after cardiopulmonary bypass in patients with diabetes is associated with increased expression of vascular endothelial growth factor and hepatocyte growth factor

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Background: Several inflammatory mediators such as vascular endothelial growth factor and hepatocyte growth factor are known to play a critical role in the regulation of vascular permeability and angiogenesis. We studied the serum levels of growth factors and gene expression profiles of genes involved in growth factor signaling in the peripheral blood of patients with and patients without diabetes following cardiopulmonary bypass and cardioplegic arrest.

Methods: Serum and total RNA were obtained from the blood samples collected from patients with diabetes and matched patients without diabetes (n = 7 patients each) who had coronary artery bypass graft before and 6 hours and 4 days after cardiopulmonary bypass/cardioplegic arrest. The cytokine panel, consisting of growth factors such as vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor, and epidermal growth factor, was quantified in patients with diabetes and patients without diabetes before and 6 hours and 4 days post–cardiopulmonary bypass/cardioplegic arrest using multiplex cytokine quantification system. cDNA microarray analysis was performed and fold-change was calculated.

Results: Length of hospitalization (10 vs 6 days; $P = .04$) and weight gain (5 vs 2.5 kg; $P = .001$) were significantly greater for patients with diabetes compared with patients without diabetes. The serum levels of vascular endothelial growth factor and hepatocyte growth factor were significantly elevated in patients with diabetes when compared with patients without diabetes before versus 6 hours post–cardiopulmonary bypass/cardioplegic arrest. In addition, significantly elevated mRNA expression of hypoxia-inducible factor-1α, cyclic adenosine monophosphate response element binding protein, and E1A binding protein p300 (more than twofold) was observed 4 days post–cardiopulmonary bypass/cardioplegic arrest exclusively in patients with diabetes.

Conclusions: The differential profile of gene and protein expression of growth factors and their related genes in patients with diabetes and patients without diabetes could be associated with increased edema and weight gain in patients with diabetes after cardiopulmonary bypass/cardioplegic arrest.

Cardiovascular disease is the leading cause of death among individuals with diabetes mellitus (DM), and ~ 34% of the patients having coronary artery bypass grafting (CABG) have been reported to have diabetes.[1] Several adverse surgical outcomes, such as higher perioperative mortality, sternal wound infections, postoperative stroke, and longer hospitalization post–cardiac surgery, have been reported in patients with DM compared with patients without DM.[2,3]

Cardiopulmonary bypass (CPB) during cardiac surgery is associated with extensive inflammatory triggers including the blood-bypass circuit interface, ischemia–reperfusion injury of heart and lungs, and operative trauma. These inflammatory signals induce the activation of microcirculation throughout the body, thus leading to multiorgan dysfunction in the immediate postoperative period.[4] The other major contributory factor for organ dysfunction after CPB is postoperative hypoxia.

Hypoxic conditions can induce a variety of genes encoding proteins including growth factors, such as vascular endothelial growth factor (VEGF), via hypoxia-inducible factor-1α (HIF-1α), which regulates vascular permeability and cell proliferation.[5] Growth factors such as insulin-like growth factor,[6] hepatocyte growth factor (HGF),[7] keratocyte growth factor,[8] transforming growth factor-β1,[9] and also the inflammatory mediators including interleukin-1 and interleukin-6[10] are known to regulate VEGF synthesis. VEGF is a multifunctional cytokine, overexpressed in hypoxia, and is known to be involved in wound healing,[11] formation of collateral vessels in ischemic tissue,[12] chronic inflammatory disorders (psoriasis, rheumatoid arthritis),[13] diabetic retinopathy,[14] and tumor growth.[15] The biologic properties of VEGF include

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
CPB = cardiopulmonary bypass
CREB = cyclic adenosine monophosphate response element binding protein
CRP = C-reactive protein
DM = diabetes mellitus
EP300 = E1A binding protein p300
HGF = hepatocyte growth factor
HIF-1α = hypoxia-inducible factor-1α
PCR = polymerase chain reaction
RMA = Robust Multichip average
VEGF = vascular endothelial growth factor

endothelial cell proliferation,[16] chemotaxis of macrophages and vascular smooth muscle cells,[17] induction of adhesion molecules (vascular cell adhesion molecule and intercellular adhesion molecule),[18] and metalloproteinase synthesis.[19] On the other hand, C-reactive protein (CRP), a potential marker for acute inflammation and a potent independent predictor for cardiovascular diseases including arteriosclerosis,[20] has been reported to down-regulate VEGF receptors on endothelial cells[21] and thus may lead to decreased angiogenesis in patients with DM. Acute systemic hypoxia can induce leukocyte extravasation into tissues via VEGF signaling followed by fluid accumulation and thus induces overall weight gain in patients after cardiac surgery.

The mechanisms contributing to the differences in the postsurgical complications observed between patients with DM and patients without DM are not well established. In this study, we evaluated serum levels of several growth factors including VEGF and HGF as well as gene expression profiles of genes involved in growth factor signaling in the peripheral blood of patients with DM and patients without DM following CPB/cardioplegic arrest. The observed differential quantitative and qualitative protein and gene expressions between patients with DM and patients without DM could provide insight into the observed differences in postoperative inflammatory state between the 2 patient populations.

MATERIALS AND METHODS

Subjects

This prospective cohort study was initiated after being approved by the Beth Israel Deaconess Medical Center Institutional Review Board/Committee on Clinical Investigations. Before enrollment, informed written consent was obtained from all patients interested in participating in the study. The 14 enrolled patients were scheduled for either elective or urgent primary CABG or valvular surgery (aortic, mitral, or both). Because all patients with DM considered for the study had elevated preoperative blood sugar levels $\geq$ 240 mg/dL, they had preoperative treatment using either an insulin infusion or subcutaneous treatment. Normoglycemic patients without DM received no further glucose treatment presurgery.

Anesthetic And Surgical Technique

Under general anesthesia, midline sternotomy was performed, and systemic heparin (100 U/kg) was administered to attain an activated clotting time > 400. The right atrium and ascending aorta were cannulated, and CPB was initiated. CPB circuit consisted of a standard roller pump, a membrane oxygenator, and a 40-μm arterial filter. The circuit was primed with crystalloid solution. CPB strategy included the use of mild hypothermia (30°C–34°C), maintenance of serum glucose levels $\leq$ 130 mg/dL with intravenous insulin injections, and α-stat pH monitoring. During CPB, insulin infusion was utilized to maintain normoglycemia, and this strategy was continued in the intensive care unit for 24 hours. Subsequently, subcutaneous insulin was utilized for postoperative glucose control. Pump blood flow was maintained between 2 and 2.4 L $\cdot$ min^{-1} $\cdot$ m^{-2} of body surface area, and mean arterial pressure was maintained between 50 and 90 mm Hg with the use of conventional vasoactive medications. Cell-Saver (Haemonetics Corp., Braintree, Mass) and cardiotomy suction were utilized. The decision to administer antifibrinolytic agents (either aprotinin or Amicar) was left to the discretion of the operating team. Cardiac arrest was induced and maintained with antegrade administration of cold-blood hyperkalemic (25 mmol/L) cardioplegic solution.

Blood Processing and RNA Extraction

Blood samples were collected in the sterile PAX tubes (Qiagen Inc, Valencia, Calif) from the central venous line after induction of anesthesia but before skin incision preoperatively and 6 hours and 4 days post–CPB/cardioplegic arrest. Blood samples were centrifuged immediately at 15,000*g* for 15 minutes, and serum/plasma samples were frozen at –80°C until the time of the assay. RNA extraction was performed using PAX gene kit (Qiagen Inc). For microarray analysis, after the quantitative and qualitative assessment of extracted total RNA, single-stranded followed by double-stranded cDNA synthesis was performed. Biotin-labeled cRNA was obtained by in vitro transcription of double-stranded cDNA using AFFI kit (Affymetrix, Santa Clara, Calif). cRNA was further purified, fragmented, hybridized overnight onto Affymetrix gene chips, washed in streptavidin followed by array scanning as previously described.[22]

Microarray Analysis

Transcriptional profiling was performed on HG-U133 plus 2.0 Affymetrix chips containing > 47,000 transcripts. The quality of the chips was assessed, and the outliers, calculated using the dChip algorithm, were eliminated from the analysis. Matrix of gene expression values was generated using Robust Multichip average (RMA) normalization statistics (John's Hopkins University, Baltimore, Md). The presence calls of transcripts obtained from Microarray system 5 in conjunction with gene expression values from RMA were used to generate a fusion model to compare the differences in gene regulation postsurgery (6 hours or 4 days) compared with pre–cardiac surgery in patients with DM versus patients without DM (n = 7 each group). Changes of more than twofold along with enhanced expression of that transcript in 50% or more of patients post–cardiac surgery for up-regulated genes or presurgery for down-regulated genes were the major criteria to determine significant differential gene regulation between the subsets ($P < .001$).

Real-Time Polymerase Chain Reaction

For real-time polymerase chain reaction (PCR) analysis, one-step reverse transcriptase–PCR reactions were performed using SYBR Green detection in the final volume of 25 μL. The specific primers for the genes of interest were designed using OligoPerfect software (Invitrogen Inc, Carlsbad, Calif). SYBR Green reactions were performed with SYBR Green PCR Master Mix (Applied Biosystems, Foster City, Calif) with added reverse transcriptase and RNAase inhibitor using ABI Prism 7000 Sequence detector system (Applied Biosystems). The following conditions were used to run the reaction: 30 minutes at 48°C, 10 minutes at 95°C for 1 cycle, and 15 seconds at 95°C and 1 minute at 60°C for 40 cycles. Relative expression levels of the genes were analyzed using 2^{-ddCT} method in comparison to the

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