Role of Stromal-Derived Factor-1α in the Induction of Circulating CD34+CXCR4+ Progenitor Cells After Cardiac Surgery

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Background—Cardiopulmonary bypass (CP/CPB) leads to an increase in circulating progenitor cells. The role of stromal-derived factor-1α (SDF-1α), a key regulator of progenitor cell mobilization, and other cytokines in this process is not clear.

Methods and Results—Peripheral blood (n=24), atrial and skeletal tissue (n=6) samples were taken from patients undergoing CP/CPB before (pre-CP/CPB), 4 hours (post-CP/CPB), and 4 days (POD4) after CP/CPB. The number of circulating CD34+CXCR4+ cells increased post-CP/CPB (442±53 versus 286±27; P=0.04 versus pre-CP/CPB), but not at POD4 (382±50; P=0.28 versus pre-CP/CPB). Plasma levels of SDF-1α increased post-CP/CPB as compared with pre-CP/CPB (3325±325 versus 2911±165 pg/mL; P=0.046) but returned to baseline at POD4 (2838±224 pg/mL; P=0.90). Plasma levels of vascular endothelial growth factor were similar post-CP/CPB (11.1±1.1 pg/mL; P<0.001) and returned to baseline at POD4 (P=0.84 versus pre-CP/CPB). The circulating CD34+CXCR4+ cells were positively correlated with plasma levels of SDF-1α early after CP/CPB (r=0.56, P<0.01), but not at other times. Protein expression of SDF-1α was elevated in the atrial myocardium after CP/CPB (9.4-fold; P=0.03).

Conclusions—Exposure to CP/CPB leads to an increase in circulating CD34+CXCR4+ progenitor cells, which is associated with increased myocardial SDF-1α expression. The numbers of CD34+CXCR4+ progenitor cells positively correlate with the plasma levels of SDF-1α post-CP/CPB, suggesting an important role of SDF-1α in progenitor cell mobilization. (Circulation. 2006;114[suppl I]:I-186–I-192.)

Key Words: cardiac surgery ■ cardiopulmonary grafting ■ cardiopulmonary bypass ■ cytokines ■ reperfusion ■ stem cells ■ stromal-derived factor

CD34 is a membrane lineage marker found on hematopoietic stem cells.1,2 A role for mobilization and trafficking of bone marrow mononuclear cells including CD34+ cells by the chemokine, stromal-derived factor-1α (SDF-1α), has been established. SDF-1α binds to its specific receptor, CXCR4, and plays a pivotal role in the mobilization and trafficking of stem cell progenitors.3 It was recently shown that increases in CD34+CXCR4+ cells in patients with acute myocardial infarction are positively correlated with plasma levels of SDF-1α.4 Subcutaneous injection of granulocyte-colony stimulating factor (G-CSF) increases the number of CD34+ cells, as well as the number of functional CXCR4 receptors on the cell membrane.5,6 The SDF-1α/CXCR4 interaction is important in the mobilization and differentiation of stem cell progenitors by G-CSF.7 In addition, vascular endothelial growth factor (VEGF) is also involved in CD34+ cell mobilization from bone marrow.8

Cardiopulmonary bypass (CP/CPB) induces an inflammatory response resulting in increases in various cytokines including VEGF and G-CSF.9,10 Furthermore, CP/CPB is associated with an increase in the number of circulating endothelial progenitors. Subeubel et al have recently shown that stem cell progenitors expressing CD34 and AC133 were increased in patients after coronary artery bypass grafting.11 Furthermore, Ruel at al. demonstrated different migratory function of progenitor cells between patients undergoing on-pump and off-pump coronary artery bypass grafting.12

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zThe role of the various cytokines induced after cardiac surgery and their effects on the mobilization of circulating progenitor cells is unclear. Further elucidation of these mechanisms may have implications for the understanding of the endogenous response to surgical injury as well as cell-based therapeutic angiogenesis. Given the critical role of SDF-1α/CXCR4 interaction in mobilization of progenitor cell, we examined the effects of CP/CPB on CD34+/CXCR4+ cells.

The purpose of the present study was: (1) determine specific changes in the number of CD34+/CXCR4+ cells after CP/CPB; (2) examine tissue and plasma levels of SDF-1α that may be involved in cell mobilization after CP/CPB; and (3) to evaluate the correlation between circulating levels of SDF-1α, VEGF, and G-CSF and the induction with CD34+/CXCR4+ cells after CP/CPB.

Materials and Methods

Human Tissue Samples
This study was approved by Institutional Review Board of Beth Israel Deaconess Medical Center, Harvard Medical School. Informed consent was obtained from patients enrolled in the study as required by the Institutional Review Board. Peripheral blood samples were taken at the three different points in 24 patients (total n = 72 samples); before skin incision (pre-CP/CPB), 4 hours after surgery (post-CP/CPB), and 4 days after surgery (POD4). Blood was collected for FACS analysis and measurement of cytokine levels. Samples of atrial tissue were harvested from the right atrial appendage before and after CP/CPB (n = 6) using a double purse string technique as previously described.13 The first specimen was taken at the time of atrial cannulation. The second specimen was collected by placing a second purse string suture below the cannulation site after weaning from cardiopulmonary bypass at the time of de-cannulation. Skeletal muscle samples were obtained from the chest wall at the time of weaning from CPB after CP/CPB, at the time of atrial cannulation. The second specimen was collected by placing a second purse string suture below the cannulation site after weaning from cardiopulmonary bypass at the time of de-cannulation. Skeletal muscle samples were obtained from the chest wall at the same time as the atrial tissue. Tissue samples were immediately frozen in liquid nitrogen and stored at −80°C for Western blotting, or fixed in 10% formalin for immunohistochemistry.

Western Blot Analysis
Tissue lysates were isolated from the atrial tissue before and after CP/CPB (n = 6) with RIPA buffer. The supernatant protein concentration was measured spectrophotometrically at a 595-nm wavelength. Sixty micrograms of total protein were fractionated by 4% to 20% gradient, SDS polyacrylamide gel electrophoresis (Invitrogen, San Diego, Calif), and transferred to PVDF membranes (Millipore, Bedford, Mass). The membrane was incubated overnight at 4°C using anti-SDF-1α antibody diluted to 1:1000 (Leinco, San Diego, Calcif). Then, the membranes were incubated for 1 hour in diluted appropriate secondary antibody anti-rabbit immunoglobulin G (Jackson Immunolab). Peroxidase was revealed by use of the diamino-benzidine-hydrogen method. The sections were then counterstained with methyl green, dehydrated, and mounted.

Flow Cytometric Analysis
The sample of the whole blood (100 µL) was stained with FITC-conjugated anti-CD34 (BD bioscience, San Jose, Calif) and phycoerythrin-conjugated anti-CXCR4 (eBioscience, San Diego, Calif) monoclonal antibodies for 30 minutes at 4°C (n = 24). The cells in each sample were double-labeled for CD34+/CXCR4+ stain. Red blood cells and platelets were subsequently lysed for 15 minutes by a erythrocyte lysis buffer (Quigen, Valencia, Calif), and the sample was centrifuged, washed twice, resuspended in phosphate-buffered saline, and analyzed by use of a FACS Caliber flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Isotype-matched FITC-conjugated and phycoerythrin-conjugated antibodies (eBioscience) were used as controls. Setting for acquisition of CD34+/CXCR4+ cells in lymphocyte population were determined using phycoerythrin-conjugated anti-CD4 (eBioscience) and FITC-conjugated anti-CD8 antibody (BD bioscience) in each sample. The number of CD34+/CXCR4+ cells was obtained as the absolute number in a total of 50 000 leukocytes. The dot plot cytometric analysis is shown in Figure 1. Leukocytes were stained by 0.15% Trypan blue and counted using the lysed sample for flow cytometry (FACS) without antibody. The number of CD34+/CXCR4+ cells in 100 µL of peripheral blood was calculated as follows: Total cells = leukocytes (cells/µL of peripheral blood) × 100 × (absolute number of CD34+/CXCR4+ cells) / 50 000.

Enzyme-Linked Immunosorbent Assay for SDF-1α in Human Plasma
Quantitative immunoassays of plasma levels of SDF-1α, VEGF, and serum levels of G-CSF were performed by the enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minn) according to the manufacturer’s instructions.

Data Analysis
All results are expressed as mean ± SEM. The results of enzyme-linked immunosorbent assay and Western blots were analyzed using 1-way repeated measurements ANOVA. The results of FACS were analyzed using Friedman test by Dunne’s multiple comparison tests. The correlations between cytokines and CD34+/CXCR4+ cells were analyzed by Spearman rank test. Statistical analyses were performed by using GraphPad Prism 4 (GraphPad SoftWare Inc, San Diego, Calif) and Statview 5.0 software (SAS Institute Inc, Cary, NC). P < 0.05 was considered significant.

Results

Patient Characteristics
The characteristics of the 24 consecutive patients who were enrolled in this study are shown in Table 1.

Increase in SDF-1α Protein Expression in Atrial Tissue After CPB
Western blot analysis was performed to evaluate SDF-1α protein expression in human tissue before and after CP/CPB (Figure 2). After CP/CPB, atrial SDF-1α protein expression was elevated 9.4 fold as compared with before CP/CPB (P = 0.03). In contrast, expression of SDF-1α in skeletal muscle, which was exposed to CPB but not CP, was undetectable.

Spatial localization of the increased protein expression of SDF-1α in atrial tissue after CP/CPB was evaluated by immunohistochemical analysis (Figure 3). Positive staining of SDF-1α was found in myocardium (Figure 3A, 3B) and...
endothelial cells of coronary arterioles (Figure 3C, 3D), venules (Figure 3E, 3F), and the endocardium (Figure 3G, 3H). The intensity of SDF-1 after CP/CPB was increased as compared with before CP/CPB.

Change in CD34/CXCR4+ Cells After CP/CPB
As shown in Figure 4A, the total number of leukocytes increased post-CP/CPB as compared with pre-CP/CPB (13.5±0.8 versus 6.8±0.4 ×1000 cells/μL; P<0.001) and remained elevated at POD4 (9.9±0.5; P<0.001 versus pre-CP/CPB).

Change of CD34/CXCR4 cells in 50 000 leukocytes is shown in Figure 4B. The number of CD34/CXCR4+ cells was unchanged (pre-CP/CPB=21.2±2.1, post-CP/CPB=16.8±2.1, POD4=19.5±2.3; P=0.09). The percentage of CD34/CXCR4+ cells in leukocytes was 0.042%. The percentage of CD34/CXCR4+ cells in the mononuclear cell population was 0.11%.

To adjust for the increase in total leukocytes, change of CD34/CXCR4+ cells in 100 μL of peripheral blood was calculated and is shown in Figure 4C. The number of CD34/CXCR4+ cells in the lymphocyte population significantly increased after CP/CPB as compared with pre-CP/CPB (442±53 versus 286±27; P=0.04). However, this increase was not evident at POD4 (382±50; P=0.28 versus pre-CP/CPB). These results indicate that elevation in the number of total leukocytes contributes to the increase in circulating CD34/CXCR4+ cells after CP/CPB.

Changes in Cytokine Levels in Plasma and Serum After CP/CPB
To investigate the effects of cytokines on the mobilization of progenitor cells, we measured levels of SDF-1α, VEGF, and G-CSF, which are involved in progenitor cell mobilization, homing, and trafficking. As shown in Figure 5A, plasma levels of SDF-1α increased post-CP/CPB as compared with pre-CP/CPB (3325±325 versus 2911±165 pg/mL; P=0.046).
but returned to baseline at POD4 (2838 ± 224 pg/mL; *P* = 0.90 versus pre-CP/CPB). As shown in Figure 5B, plasma levels of VEGF were unchanged early post-CP/CPB (*P* = 0.90) but increased significantly at POD4 (220 ± 40 versus 134 ± 26 pg/mL; *P* = 0.04 versus pre-CP/CPB). As shown in Figure 5C, serum levels of G-CSF were elevated 24-fold post-CP/CPB (265 ± 42 versus 11 ± 1 pg/mL; *P* < 0.001 versus pre-CP/CPB) and returned to baseline at POD4 (34 ± 18 pg/mL; *P* = 0.84). In summary, plasma SDF-1α and serum G-CSF levels increase early after CP/CPB, whereas plasma VEGF levels are elevated at POD4.

### Correlation Between CD34+CXCR4+ Cells and Increased Cytokine Levels

Correlations between the number of total CD34+CXCR4+ cells and cytokine levels of SDF-1α, VEGF, and G-CSF were evaluated at pre-CP/CPB, post-CP/CPB, and POD4, respectively. The number of total CD34+CXCR4+ cells correlated moderately with plasma levels of SDF-1α at post-CP/CPB (r = 0.56, *P* < 0.01) (Figure 6B). Although there was a trend toward a correlation at pre-CP/CPB (r = 0.29, *P* = 0.16), this did not reach statistical significance. There was no significant correlation at POD4 (*P* = 0.24). Total number of CD34+CXCR4+ cells did not correlate with plasma levels of VEGF or serum levels of G-CSF.

### Discussion

Recently, considerable attention has been devoted to the role of accumulating stem cell progenitors toward injured tissue such as thrombus, ischemic zones, and sites of arterial injury. It has been hypothesized that these cells may be involved in tissue repair after injury as well as angiogenesis. Cardiac surgery using CP/CPB results in a controlled ischemia-reperfusion injury and in this context, CD34+CXCR4+ cells, in response to enhanced SDF-1α expression, may be involved in myocardial tissue repair. Given that SDF-1α/CXCR4 interaction is a critical factor in mobilization and homing of progenitor cell, the findings of this study suggest that SDF-1α plays an important role in the mobilization of progenitor cells after CP/CPB. We found that protein expression of SDF-1α is significantly increased in the myocardial tissue subjected to ischemia-reperfusion (CP) but not in skeletal muscle that is exposed to CPB alone. Second, circulating CD34+CXCR4+ cells were increased early but
This increase in circulating CD34+/CXCR4+ cells was associated and significantly correlated with plasma levels of SDF-1. Last, although CP/CPB resulted in the elaboration of other cytokines, i.e., VEGF and G-CSF, these were not correlated with circulating levels of CD34+/CXCR4+ cells.

SDF-1α is produced in response to tissue damage and plays an important role in the mobilization of CXCR4+ cells. Several cell types such as cardiomyocytes, muscle-derived fibroblasts, and endothelial cells secrete SDF-1α. In addition, ischemia and hypoxia can induce SDF-1α expression. In this study, we demonstrated that SDF-1α expression is increased in atrial myocardium and plasma early after CP/CPB. The protein expression of SDF-1α was not found in skeletal muscle. This suggests that the increased expression of SDF-1α is associated with cardioplegic ischemia-reperfusion rather than cardiopulmonary bypass alone. Protein expression of SDF-1α was found in cardiomyocytes and endothelial cells of arterioles, venules, and the endocardium, which may increase the ability of these tissues to bind circulating progenitor cells expressing CXCR4. Thus, locally induced SDF-1α expression may contribute to the mobilization of circulating CD34+/CXCR4+ cells.

In this study, we demonstrated that the number of CD34+/CXCR4+ cells correlate with the plasma levels of SDF-1α at post-CP/CPB. However, this correlation was not evident at pre-CP/CPB or POD4, suggesting that induction of progenitor cells and key cytokines occurs within the first few hours after the injury. G-CSF mobilizes stem cell progenitors from bone marrow and upregulates intracellular CXCR4 on the cell surface resulting in presentation of the CXCR4 receptor.

Subcutaneous G-CSF treatment for 5 days increased the circulating number of progenitors >10-times in patients with coronary artery disease. We found that the increased number of CD34+/CXCR4+ cells were limited and did not correlate with the elevated serum levels of G-CSF. The endogenous increase in G-CSF induced by CP/CPB was not enough to mobilize the number of progenitors to peripheral blood as compared with the exogenous treatment by G-CSF. Thus, the circulating CD34+/CXCR4+ cells may respond preferentially to the increased plasma levels of SDF-1α rather than G-CSF after CP/CPB. Scheubel et al have shown that the increase in CD34+/AC133+ cells peaks at 6
hours after CP/CPB, decreases at 1 day and increases again 3 days after CP/CPB. They also suggested that the decline in cell number may be due to cell accumulation in injured tissue. These lines of evidence indicate the potentially important role of locally induced SDF-1α at the site of ischemia and the increased number of circulating CD34+CXCR4+ cells early after CP/CPB.

Plasma levels of VEGF are persistently elevated and correlated with the number of circulating CD34+ cells in patients after ischemic stress. In patients with acute myocardial infarction, plasma levels of VEGF remained increased at 7 days after onset and correlated with CD34+ cells.5,10 In patients undergoing cardiac surgery, increased levels of VEGF were found at 3 days after CP/CPB.11 In agreement with previous results, we found that plasma levels of VEGF were elevated 4 days after surgery. However, the sources of plasma VEGF are unclear. Recent publications have demonstrated that cultivated mononuclear cells and endothelial progenitors release VEGF in ex vivo experiments, but mature endothelial cells do not.20,21 Interestingly, in in vivo experiments, cultured CD34+ cells injected into ischemic myocardium secrete VEGF as well as increase myocardial capillary density.22 We have previously shown that CP/CPB induces VEGF protein expression in atrial myocardium.23 In the present study, we have demonstrated that the plasma levels of VEGF increase at 4 days after surgery, and that they are not correlated with the number of circulating CD34+CXCR4+ cells after CP/CPB.

Progenitor cells make up a small fraction of circulating mononuclear cells. The ratio of total CD34+CXCR4+ cells in mononuclear cells in our study was 0.11%. Other groups have reported similar numbers of specific CD34+ populations in patients with coronary artery disease. Specifically, Leone et al reported that the number of CD34+ cells contained in 100 μL of peripheral blood in patients with stable angina is 341±56.24 In addition, the percentage of CD34+AC133+, AC133+CXCR4+ cells contained in mononuclear cells taken from peripheral blood in patients were 0.121% and 0.165% respectively.18 Thus, the proportion of circulating CD34+CXCR4+ progenitor cells in our study is concordant with previously published data.

Limitations
Although we showed increased number of CD34+CXCR4+ cells after CP/CPB, further studies are needed to evaluate differences in progenitor cell characteristics, expression of other lineage markers, as well as progenitor cell function. It has been reported that other cytokines, eg, placenta growth factor, stem cell factor, and IL-6, are involved in stem cell mobilization from bone marrow and/or upregulation of CXCR4 expression of CD34+ cells. The relationship between these cytokines and CD34+CXCR4+ cells should be evaluated in future studies.

Conclusions
The increase in circulating CD34+CXCR4+ progenitor cells after cardiac surgery is associated with and significantly correlated with plasma levels of SDF-1α. Furthermore, local induction of SDF-1α in myocardial tissue may be responsible for progenitor cell mobilization to injured tissue after CP/CPB. These results suggest an important role for SDF-1α in progenitor cell mobilization.

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Disclosures
None.

References


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