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Circulating Levels of Endothelial Progenitor Cell Mobilizing Factors in The Metabolic Syndrome

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Abstract

Endothelial progenitor cells (EPCs) appear to be an emerging biomarker of vascular health. However, there is scanty data on EPC biology and its mobilizing factors in the Metabolic syndrome (MS). In this study, we assayed EPC mobilizing factors including granulocyte colony stimulating factor (G-CSF), stem cell factor/c-kit ligand (SCF), vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1 (SDF-1) levels in patients with MS (n = 36) and age and gender-matched controls (n = 38). There was a significant reduction in G-CSF levels (83% decrease) in the patients with MS. Also, there was a decrease in SCF and SCF soluble receptor levels. However, there was no significant difference in SDF-1 levels and paradoxically, VEGF levels were increased consistent with resistance. In conclusion, in addition to progenitor cell exhaustion as a mechanism for the decrease in EPC in patients with MS, we suggest that they also have a mobilization defect as manifest by decreased levels of G-CSF and SCF resulting in a decrease in EPC.

Keywords

Progenitor cells; endothelium; metabolic syndrome; prediabetes; mobilizing factors

Introduction

Endothelial Progenitor Cells (EPC), defined by dual positivity of CD34 and KDR, have been shown to correlate with endothelial function, risk factors for coronary artery disease (CAD), cardiovascular disease (CVD) severity and incident cardiovascular events (CVE) ^{1–5}. There is scant data on EPC number and functionality in metabolic syndrome (MS) ⁶. Two studies on patients with MS without manifest CVD or diabetes showed a decrease in EPC number, and the larger study (MS n=46) also documented impaired EPC functionality ^{7,8}. However, they contrasted with respect to quantitative levels of CD34+ progenitor cells (PC), since the smaller study in obese males (n=19) showed no significant decrease whilst the larger study,

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conducted in both males and females, showed a significant decrease in CD34+ cells in MS patients. This PC decrease accord with that reported by Fadini et al. in MS patients with diabetes or peripheral arterial disease (PAD)⁹ and argues for bone marrow exhaustion as one explanation for the decrease in CD34+KDR+ EPCs. In the study of obese males there was a decrease in plasma concentration of the mobilizing factor stem cell factor/c-kit ligand (SCF), but not of vascular endothelial growth factor (VEGF). These preliminary data point to a possible defect in bone marrow mobilization of EPC in MS patients. Due to the paucity of data on EPC mobilizing factors⁶ and their critical role in determining EPC status, we undertook a more comprehensive study and report on granulocyte colony stimulating factor (G-CSF), SCF, VEGF and stromal cell-derived factor-1 (SDF-1) levels in patients with MS compared to matched controls, as modulators of PC and EPC mobilization.

Method

All subjects were recruited from Sacramento County through fliers and advertisements in the newspaper. Subjects (age 21–70 years) with MS (n=36) and healthy controls (n=38) were studied. MS was defined using the criteria of the National Cholesterol Education Program Adult Treatment Panel-III¹⁰. Control subjects needed to have ≤ 2 features of MS and not be on blood pressure (BP) medications. Other selection criteria have been published previously⁸. None of the subjects had diabetes, cardiovascular disease or were on medications known to affect EPC biology¹¹. This protocol was approved by the Institutional Review Board at University of California Davis.

After history and physical examination, fasting blood was obtained. Enumeration of peripheral blood PC and EPCs were performed by flow cytometry as described previously⁸. Cells positive for both CD34 and KDR were characterized as EPCs. Also, the number of PC was quantified as CD34 positive cells. We have previously shown decrease in PC and EPC in this cohort⁸.

Plasma SDF-1 and VEGF levels were measured by sandwich ELISA according to the manufacturer's protocol (R&D Systems) and expressed in pg/mL. SCF, SCF-sR (SCF-soluble receptor) and G-CSF levels were measured in serum samples by ELISA according to the manufacturer's protocol (R&D Systems). SCF-sR levels were expressed in pg/mL and SCF and G-CSF levels were expressed in pg/mL. Inter-assay coefficient of variation (CV) for all the ELISAs was $< 10\%$ except for G-CSF with a CV of 14% . HsCRP levels were measured in serum using the Beckman Dxl⁸.

Data were expressed as mean $\pm$ SD or, for skewed variables as median and interquartile range. Log transformations were applied to skewed data prior to parametric analyses. Comparisons between the control and MS groups were made with two-sample t-tests. Spearman's rank correlation coefficients were computed to assess the association between mobilizing factors and both PC and EPC numbers. Data were analyzed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

The 2 groups were matched for age and gender. All metabolic features including homeostasis model assessment (HOMA) and hsCRP were significantly increased in patients with MS and HDL-C levels were significantly decreased (Table 1). Also, both levels of progenitor cells (PC) and endothelial progenitor cells (EPC) were significantly decreased in MS patients ($p<0.05$).

With respect to the mobilizing factors, the most significant difference was seen with G-CSF levels with an 83% decrease in patients with MS (Figure 1). Also levels of both SCF and

SCF-sR were significantly decreased. However SDF-1 levels were not different between the 2 groups (Table 2).

Levels of VEGF were significantly increased. Correlations were undertaken between mobilizing factors and CD34+ PC or CD34+KDR+ EPC numbers, both of which we have previously reported to be decreased in MS patients⁸. The only significant correlations were between EPC and G-CSF ($r=0.26$; $p=0.041$) and between PC and VEGF($r=0.42$; $p=0.044$). There were no significant correlations between mobilizing factors and HOMA and hsCRP levels.

Discussion

We and others have previously shown that PCs and EPCs are decreased in subjects with MS^{6–9}. This finding was further confirmed in this study with a smaller sample size. This observation might have profound implications in terms of cardiovascular risk, as both CD34+¹² and CD34+KDR+ cells^{4,5} predict incident CVE and regulate cardiovascular homeostasis. However, the causes of (E) PC pauperization in MS remain unclear. Herein, we expand our previous observations on decreased (E)PC level and impaired functionality⁸ and suggest that an imbalance in the levels of mobilizing factors may be responsible in part for reduced (E)PC. Striking was the reduction of G-CSF associated with MS, which was accompanied by low levels of SCF. The reduced concentration of SCF-sR argues against a decoy effect by soluble c-kit generated by cleavage of the membrane isoform¹³ and suggests a reduced expression of SCF. Interestingly, G-CSF and SCF are known to synergize in inducing bone marrow PC mobilization¹⁴ and plasma levels of SCF have been also taken to represent a measure of PC mobilization¹⁵. Given that they are both produced by, among other cells, the endothelium, it is possible that this represents a novel feature of endothelial dysfunction associated with MS. The paradox of increased VEGF levels may reflect VEGF post-receptor resistance, as seen in diabetes and argues for VEGF functional impairment since EPC numbers were significantly decreased^{16–18}. In this study, the direct PC/VEGF and the inverse EPC/G-CSF correlations suggest that an imbalance in VEGF and G-CSF in MS patients may be responsible for defective EPC generation and mobilization from bone marrow PC. This hypothesis is supported by observation that VEGF does not stimulate bone marrow PC in the absence of G-CSF¹⁹. Thus, we hypothesize that since G-CSF, SCF and SCF soluble receptor are significantly decreased coupled with a functional deficiency of VEGF (resistance) that inspite of normal levels of SDF, patients with MS have an imbalance of mobilizing factors supporting a mobilizing defect. In future studies, we will examine VEGF resistance in monocytes by studying VEGF receptors (VEGF R1 and VEGF R2)²⁰, post-receptor signaling and also the status of the SDF receptor CXCR4²¹ on EPCs in MS. Noteworthy, in the present report, the imbalance of mobilizing factors was found in MS without diabetes and CVD, indicating that it may have a role early in the natural history of the disease.

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Figure 1. EPC Mobilizing Factors in Metabolic Syndrome

SCF and G-CSF levels were measured in serum samples of Controls and MS patients by ELISA as described in Methods. * $p < 0.001$ compared to controls

Table 1

Baseline Characteristics

Variable	Controls (n=38)	Metabolic Syndrome(n=36)
Age (year)	49 ± 12	53 ± 11
Waist circumference (inches)	36 ± 6	43 ± 5 *
Male: Female	7:31	10:26
Blood Pressure		
Systolic (mmHg)	118 ± 13	132 ± 12 *
Diastolic (mmHg)	73 ± 8	82 ± 10 *
Fasting Glucose (mg/dl)	89 ± 7	101 ± 11 *
Total Cholesterol (mg/dl)	188 ± 32	200 ± 27
HDL-Cholesterol (mg/dl)	54 ± 14	40 ± 11 *
LDL-Cholesterol (mg/dl)	119 ± 26	130 ± 20
Triglycerides (mg/dl) ^I	72	140 *
Insulin (mIU/mL)	3.5 ± 0.7	7.5 ± 1.2 *
HOMA-IR	1.8 (0.91,2.9)	4.1 (2.5,6.3) *
hsCRP (mg/L) ^I	1.4	3.5 *
Progenitor Cells (CD34+, mfi)	12.1 (9.9, 21.4)	8.4 (6.5, 11.7) *
EPC (CD34+/KDR+, mfi)	5.1 (3.3,8.9)	3.6 (2.5,6.7) *

Unless otherwise noted, data were mean ± SD;

^I Data were medians;

* p<0.05,

** p<0.01,

*** p<0.001, compared to Controls

Abbreviations: EPC: endothelial progenitor cells; HDL-High density lipoprotein; HOMA_IR: Homeostasis Model Assessment of Insulin Resistance; hsCRP: High sensitivity C-reactive protein; LDL-Low density lipoprotein

Table 2

Endothelial Progenitor Cell Mobilizing Factors

Variable	Controls (n=38)	Metabolic Syndrome (n=36)
SCF-sR (pg/mL)	20 ± 6	15 ± 4 **
SDF-1 α (pg/ml)	2.2 ± 0.4	2.2± 0.4
VEGF (pg/mL)	41 ± 29	85 ± 44 *

Data were mean $\pm$ SD.

*
p<0.05,

**
p<0.01, compared to Controls

Abbreviations: SCF-sR: stem cell factor/c-kit ligand soluble receptor; SDF: stromal cell-derived factor; VEGF-vascular endothelial growth factor