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Reduced circulating endothelial progenitor cell number in healthy young adult hyperinsulinemic men

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KEYWORDS
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Abstract *Background and aims:* The number of Endothelial Progenitor Cells (EPCs) is considered a novel marker of cardiovascular (CV) disease. It is not clear which are the main determinants of EPC number in apparently healthy subjects in the absence of overt clinical CV or metabolic abnormalities. We evaluated the main clinical determinants of EPC levels in a population of healthy subjects with normal glucose tolerance.
Methods and results: EPC number was determined in 122 healthy subjects (73M/49F;36.6 ± 8yrs). Blood samples were collected to test biochemical variables. OGTT was performed and insulin resistance/compensatory hyperinsulinemia was defined according to fasting plasma insulin (FPI) levels. EPCs were identified as cells co-expressing CD133/CD34/KDR antigens by flow-cytometry. $CD133^+/KDR^+$ count inversely correlated with BMI (rho = −0.18;$p < 0.05$), waist circumference (−0.2;<0.05), diastolic (−0.23;<0.01) and systolic blood pressure (−0.21;<0.05), uric acid (−0.24;<0.005), PAI-1 (−0.197; <0.05) and FPI (−0.2;<0.05) and

Abbreviations: APC, allophycocyaninl; BMI, Body Mass Index; CV, cardiovascular; CVD, cardiovascular disease; CVs, coefficients of variation; EPCs, endothelial progenitor cells; FITC, fluorescein isothiocyanate; FPI, fasting plasma insulin; HDL, high density lipoprotein; hsCRP, high sensitive C-reactive protein; IGT, impaired glucose tolerance; IR/CH, insulin resistant/compensatory hyperinsulinemia; IS, insulin sensitive; KDR, kinase insert domain receptor; OGTT, oral glucose tolerance test; PAI-1, Plasminogen activator inhibitor-1; PBS, phosphate-buffered saline; PE, phycoerythrin.

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directly correlated with HDL cholesterol (0.182;<0.05). $CD34^+/CD133^+/KDR^+$ count inversely correlated with uric acid (−0.28;<0.005) and FPI (−0.2;<0.05). EPC number was lower in males ($p < 0.05$) and gender was the only independent predictor of EPC count ($p < 0.05$). By dividing the population in four subgroups based on gender and insulin resistance, $CD133^+/KDR^+$ levels were lower in insulin resistant compared to insulin sensitive males ($p < 0.05$) with no differences in females.

Conclusion: The male gender is an independent predictor of low EPC levels in healthy subjects. This might contribute to explaining the higher CV risk in males compared to pre-menopausal age-matched females. In this study a reduced EPC number seems to be associated with insulin resistance in male subjects.

Introduction

Human peripheral blood contains circulating cells named endothelial progenitor cells (EPCs) that are a subset of circulating mononuclear cells derived from the bone-marrow [1]. EPCs have the ability to differentiate into mature endothelial cells and contribute to vessel homeostasis and repair following endothelial damage [1,2]. EPC number is lower in the presence of classical risk factors for cardiovascular (CV) disease and it has been reported to be lower in males compared to premenopausal age-matched females [3,4]. Reduced EPC number has also been related to the development of atherosclerosis and has been shown to predict CV risk [4,5]. In humans, the number of circulating EPCs is reduced also in the presence of clinical features of insulin resistance. Insulin resistance is associated with a cluster of metabolic and haemodynamic abnormalities that are well known risk factors for cardiovascular disease (CVD) leading to increased CV morbidity and mortality [6,7]. Unselected groups of patients with diabetes are reported to have reduced circulating EPC number and impaired EPC function compared to non-diabetic subjects with or without CV disease [8,9]. Circulating $CD34^+$ progenitor cell count has been found to be negatively correlated with CV risk factors and the clustered metabolic syndrome components [10].

To date, it is not clear which are the main determinants of EPC number in apparently healthy subjects in the absence of overt clinical CV or metabolic abnormalities.

We therefore conducted a study to evaluate which are the main clinical determinants of EPC levels, measured with flow-cytometry as cells expressing CD133/KDR and CD133/CD34/KDR surface antigens, in a population of healthy subjects with normal glucose tolerance.

Methods

Study population

Sample population consisted of young healthy adults recruited from the offspring of the longitudinal observational Barilla Study follow-up cohort (1993—1995). The Barilla Study is an epidemiologic survey which has investigated 325 apparently healthy subjects since 1981, studying the potential relationship between insulin resistance and CVD [7].

Study protocol was approved by the local Ethics Committee. Caucasian subjects from both genders between 18 and 60 years of age were enrolled. The exclusion criteria were previous history of CV events, impaired glucose tolerance (IGT), diabetes, medical history of neoplastic disease, acute or chronic illnesses and alcohol intake >80 g/day. Women were eligible only if premenopausal and not on estroprogestin pill. Assessment of women in the study was planned in the follicular phase of the menstrual cycle phase. Out of 140 subjects, 126 were eligible to participate in the study and signed the consent form.

All participants had their medical history and anthropometric parameters recorded: age, gender, family history for diabetes and CVD, smoking habits, Body Mass Index (BMI), waist circumference, systolic and diastolic blood pressure.

Venous plasma samples were drawn after an overnight fasting and abstention from smoking and coffee/tea drinking for the last 12 h. Blood was collected for the determination of EPCs, lipid and hepatic profile (ALT, AST, γ − GT), creatinine, uric acid, Plasminogen Activator Inhibitor-1 (PAI-1) and high sensitive C-reactive protein (hsCRP). An oral glucose tolerance test (OGTT)(75 g) was performed in all subjects and plasma glucose and serum insulin levels were measured at baseline, 30, 60 and 120 min. Glucose tolerance was defined according to the American Diabetes Association Criteria [11]. Insulin resistance/compensatory hyperinsulinemia, as a reliable surrogate marker of insulin resistance, was defined according to fasting plasma insulin (FPI) quartiles [12—14].

Assays

Biochemical variables were assessed by a central laboratory using standard methods. Serum insulin levels were assayed with a microparticle enzyme immunoassay (IMX; Abbott Laboratories, Rome, Italy), with an intra- and inter-assay coefficients of variation (CVs) of 3.0 and 5.0%, respectively. PAI-1 was measured using an ELISA kit (Hyphen Biomed, Neuville-Sur-Oise, France) (intra- and inter-assay CVs 4.0% and 6.0% respectively); hsCRP was measured using an ELISA kit (ICN Pharmaceuticals, Orangeburg, NY, USA) (intra- and inter-assay CVs 2.3 and 2.5%, respectively).

Quantification of circulating endothelial progenitor cells

Circulating EPCs were analysed by flow-cytometry (FACS Calibur, Becton Dickinson Biosciences, Franklin Lakes, NJ,