Letter to the Editor

Exposure to extreme climatic environments reduces circulating endothelial progenitor cells

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The human body's response to adverse environmental conditions and recovery is of great interest to understand mechanisms of physiologic adjustment in the cardiovascular system.

Circulating endothelial progenitor cells (EPCs) are likely to play an important physiological role by maintaining vascular integrity [1], and thus serve as a reservoir device that can replace a dysfunctional endothelium, with a protective role even in the earliest stages of the atherogenetic process and other cardiovascular diseases [2]. Decreased availability of EPCs is recognized as an important mechanism for the occurrence of vascular disease and dysfunction [3]. It has been demonstrated that baseline blood levels of EPCs are inversely correlated with the total number of cardiovascular risk factor in men without a history of coronary artery disease (CAD) [4].

In order to exert their vascular regenerative actions, EPCs are mobilized from the bone marrow into the bloodstream. The initial step of homing of EPCs to ischemic tissue involves adhesion, and transmigration occurs in response to a variety of cytokines and integrins activated by hypoxia. Stromal-derived factor (SDF-1), vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor, nitric oxide (NO) and the mobilizing factor stem cell factor/c-kit ligand (SCF) are strong chemo-attractive factors to EPCs [5,6]. In particular, plasma levels of SCF have been taken to represent a measure of progenitor cell mobilization [6]. Moreover, Endothelin-1 (ET-1) decreases eNOS expression and could therefore play an important role in EPC mobilization. In a recent study it was demonstrated that high ET-1 levels predict a lower EPC mobilization after 1 week from admission for ST-elevation myocardial infarction (STEMI) patients [7]. Another molecule which seems to be involved in EPC mobilization is the soluble intercellular adhesion molecule-1 (sICAM-1), a marker of endothelial cell activation. Indeed, up-regulation of sICAM-1 in the

ischemic muscle was shown to associate with enhanced EPC recruitment to ischemic limbs [8].

Up to now the response of EPCs in extreme conditions is still unknown.

We aim to evaluate the response of circulating EPCs, SCF, SDF-1, ET-1 and sICAM-1 to extreme temperature in volunteers participating 2 months Antarctic mission, according to the Italian Antarctic Research Programme (PNRA, Programma Nazionale di Ricerche in Antartide.

Complete clinical and cardiovascular assessments including ECG and echocardiography were performed in 6 volunteers (4 male, 2 female; mean age, 38.5 ± 8.6 years), before (time 0, t0) and after (time 1, t1) Antarctic mission. The presence of cardiovascular risk factor, including tobacco smoking, diabetes mellitus, hypercholesterolemia, and hypertension, was performed at the time of enrolment. Informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committees.

Circulating EPCs, defined by the expression of surface markers CD133/CD34/VEGFR2, were evaluated by flow cytometry analysis at t0 and t1. Briefly, 100 ml of fresh peripheral blood was incubated with 5 µl PerCP-labeled CD34, 5 µl AlexaFluor 647-labeled VEGFR2 and 10 µl PE-labeled CD133 conjugated monoclonal antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany) in the dark at 4 °C, according to the manufacturers' instruction. Red cells were lysed with a 1 × BD FACS lysis solution (BD Pharmigen, UK) and incubated for 5 min at room temperature. Validation of the assay was performed adopting the gating strategy defined by the International Society of Haematotherapy and Graft Engineering (ISHAGE) guidelines [9]. Quantitative fluorescence analysis was performed with a FACS-Calibur instrument and data were processed using Cell Quest Software (BD Pharmigen, UK).Each analysis included at least 500,000 events. The number of EPCs was expressed as absolute number of cells per milliliter of blood. Plasma levels of SCF, the α isoform of SDF-1 (SDF-1α), ET-1 and sICAM-1 were measured using a specific Enzyme Linked Immunosorbent Assay (ELISA) kit (R&D Systems, USA), according to the manufacturer's instruction.

Comparisons between groups were performed using paired *t* test.

Results are considered significant at a P-value<0.05.

All volunteers were free of cardiovascular risk f actors. None of them showed differences in clinical or cardiovascular assessment after the mission (t1) compared to t0 (data not shown). However, circulating EPC numbers were significantly reduced at t1 compared to t 0 (257 ± 77 vs 92 ± 29; P<0.05), as shown in Fig. 1.

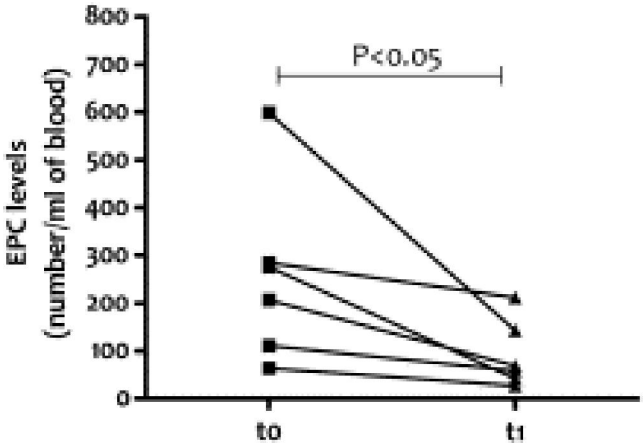

Fig. 1. EPC levels in subjects (n = 6) before (t0) and after (t1) Antarctic mission.

As shown in Fig. 2, plasma levels of SC F were significantly increased at t1 compared to t0 (P<0.0001), while plasma levels of SDF-1α did not differ between t0 and t1. Plasma levels of both ET-1 and sICAM-1 showed a positive trend at t1 compared to t0 (P = 0.0968 and P = 0.0897, respectively).

Based on these results, we hypothesize that the reduction observed in EPC levels after the Antarctic mission can be due to the fact that extreme low temperature increased plasma levels of SCF which, in the presence of an endothelial dysfunction evidenced by the trend in the increase of both ET-1 and sICAM-1, can exert their chemo-attractive function on EPCs, thus reducing their circulating levels. These preliminary results deserve attention since even though these subjects do not have any clinical evidence of cardiovascular damage, the presence of reduced levels of EPCs could be an early index of cardiovascular subclinical damage that should be monitored strictly.

Future studies in a larger population of subject exposed to extreme conditions are required to support our hypothesis and to identify determinants of mobilization as well as to prospectively define the prognostic importance of reduced levels of EPCs.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

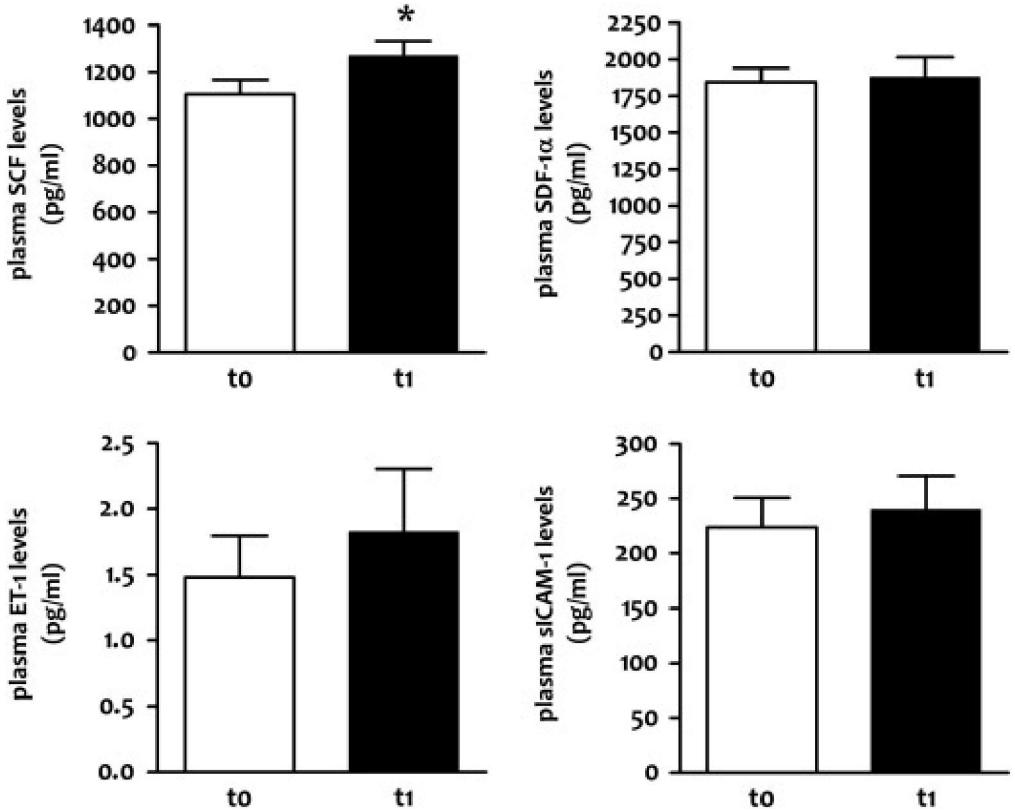

Fig. 2. Plasma SCF, SDF-1 (SDF-1α), ET-1 and sICAM-1 levels before (t0) and after (t1) Antarctic mission of n=6 subjects. *P<0.0001.

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