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Short Communication

Progenitor cells are mobilized by acute psychological stress but not beta-adrenergic receptor agonist infusion

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ABSTRACT

Objectives: Stimuli that activate the sympathetic nervous system, such as acute psychological stress, rapidly invoke a robust mobilization of lymphocytes into the circulation. Experimental animal studies suggest that bone marrow-derived progenitor cells (PCs) also mobilize in response to sympathetic stimulation. Here we tested the effects of acute psychological stress and brief pharmacological β-adrenergic (βAR) stimulation on peripheral PC numbers in humans.

Methods: In two studies, we investigated PC mobilization in response to an acute speech task ($n = 26$) and βAR-agonist (isoproterenol) infusion ($n = 20$). A subset of 8 participants also underwent the infusion protocol with concomitant administration of the βAR-antagonist propranolol. Flow cytometry was used to enumerate lymphocyte subsets, total progenitor cells, total haematopoietic stem cells (HSC), early HSC (multi-lineage potential), late HSC (lineage committed), and endothelial PCs (EPCs).

Results: Both psychological stress and βAR-agonist infusion caused the expected mobilization of total monocytes and lymphocytes and $CD8^+$ T lymphocytes. Psychological stress also induced a modest, but significant, increase in total PCs, HSCs, and EPC numbers in peripheral blood. However, infusion of a βAR-agonist did not result in a significant change in circulating PCs.

Conclusion: PCs are rapidly mobilized by psychological stress via mechanisms independent of βAR-stimulation, although the findings do not exclude βAR-stimulation as a possible cofactor. Considering the clinical and physiological relevance, further research into the mechanisms involved in stress-induced PC mobilization seems warranted.

1. Introduction

Progenitor cells (PCs) comprise an heterogeneous population uniquely capable of both self-renewal and multi-lineage differentiation (Weissman, 2000). They replenish specialized somatic cells and maintain the normal turnover of regenerative tissues and organs, such as the blood and skin. PCs generally reside in the bone marrow, with a small number continually migrating into the circulation and tissues (Mazo et al., 2011). Enhanced mobilization of endothelial PCs (EPCs) into the blood has been associated with improved endothelial function and repair (Foresta et al., 2010). Conversely, low circulating PC number and reduced PC function are associated with cardiovascular disease and mortality. Likewise, successful reconstitution of the obliterated haematopoietic system in chemotherapeutic or radiation treated patients is critically dependent on mobilizing at least 2×10^6 HSCs per kg body mass from the donor (Winkler and Levesque, 2006). Thus, there is clinical potential for methods that could aid mobilization of stem cells.

The bone marrow receives dense sympathetic innervation (Elenkov et al., 2000). Animal studies show that sympathetic nervous system activation induces the release of PCs into the blood, that this mobilization can be replicated by administration of a

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β_2AR-agonist, and that increased circulating PC numbers correlate with circadian sympathetic oscillations (Katayama et al., 2006; Spiegel et al., 2007; Mendez-Ferrer et al., 2008; Dar et al., 2011). Further, both murine and human PCs express functional adrenergic receptors (Muthu et al., 2007; Spiegel et al., 2007). In humans the number of circulating PCs is increased by sympathetic stimuli such as exercise and acute myocardial infarction, and can be reduced by treatment with βAR-antagonist (Barrett et al., 1978; Shintani et al., 2001; Bible et al., 2014).

The above observations are remarkably similar to those reported for lymphocytes, which are likewise mobilized during sympathetic activation via stimulation of β_2AR expressed on these cells (Benschop et al., 1996; Dimitrov et al., 2010). Stress and beta-adrenergic induced lymphocytosis is a rapid response, observable within minutes. Considering the strong resemblance between observations and mechanisms of PC and lymphocyte mobilization, the present study tested if acute stress and brief infusion of the βAR agonist isoproterenol may promote rapid mobilization of HSC and EPC into peripheral blood in humans.

2. Methods

2.1. Participants

The stress study was performed at the University of Birmingham (UoB) and the infusion study at the University of California San Diego (UCSD). Methods and procedures were rigorously standardized to ensure comparability between results obtained between each site (further detailed below). All participants reported to be in good health and were non-medicated with exception of the contraceptive pill. Volunteers were instructed not to engage in strenuous physical exercise, to refrain from consuming alcohol or non-prescription drugs 24 h before their experimental session, and to abstain from smoking and caffeine on the day of the experiment. Participants provided informed consent and study protocols were approved by the appropriate institutional review boards (UoB or UCSD).

2.2. Psychological stress study

2.2.1. Procedure

Twenty-six volunteers (mean age 31.5 years, SD ± 8.0; 12 female) gave informed consent and had: (1) electrodes for electrocardiography (ECG) and impedance cardiography (ICG) placed; (2) an intravenous cannula (Becton–Dickinson, Oxford, UK) inserted; and, (3) an occluding cuff placed for systolic (SBP) and diastolic (DBP) blood pressure measurements. While seated in a comfortable upright position, participants filled out questionnaires and engaged in leisure reading. After 20 min, a baseline blood sample was obtained and the laboratory stressor was initiated.

2.2.2. Public speaking task

Participants performed two back-to-back speeches as previously described (Bosch et al., 2009). A blood sample was obtained 13 min into the stress task. A final blood sample was obtained after 15 min of recovery.

2.2.3. Cardiovascular assessment

Cardiac sympathetic and vagal control were assessed as previously described (Bosch et al., 2003). ECG and ICG signals were assessed using six Ag–AgClspot-electrodes (AMI type 1650-005, Medtronic) and the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) device (Willemsen et al., 1996). Average heart rate (HR) and cardiac pre-ejection period (PEP) over 6 min was computed during baseline, both tasks, and during the recovery period. PEP was used to index changes in cardiac sympathetic drive, whereas heart rate variability, computed as Root Mean Square of Successive Difference (RMSSD), was used to index changes in cardiac vagal tone.

2.3. βAR-agonist infusion study

2.3.1. Procedures

Twenty volunteers (Mean age 35.9 years, SD ± 9.3; 8 female) were infused with the βAR-agonist isoproterenol according to a standardized protocol (Mills et al., 2000). Body surface area (BSA) was used to standardize the βAR-agonist infusion rate. Participants rested in a semi-supine position for 15 min following placement of: (1) two intravenous cannulas (Becton–Dickinson); (2) three spot ECG electrodes; and, (3) an occlusion cuff.

The βAR-agonist was infused at incremental rates (0.1 μg, 0.5 μg and 1 μg/min/1.73 m^2 BSA for 5 min each) for 15 min until the participant's heart rate had increased by ~20 beats per minute (bpm), to correspond with the level of cardiovascular activity induced by the speaking task. The final infusion rate was maintained for 10 min. On average, the maximal dose reached 1 μg/min/1.73 m^2 BSA. Blood was taken immediately prior to infusion ('baseline') and during the final minutes of the infusion. ECG, heart rate and blood pressure were monitored throughout the procedure.

2.3.2. βAR-antagonist

A sub-group of 8 participants (Mean age 34.6, SD ± 11.5 years, 2 female) underwent the infusion twice following a 5-day course of either of 80 mg of the non-selective βAR-antagonist propranolol or placebo. Condition was counter-balanced, separated by at least 7 days and single-blinded.

2.4. Questionnaires

Affective responses to the speaking task were assessed using the short-form of the Profile of Mood States (POMS) (McNair et al., 1992). Participants completed the POMS at baseline, immediately post-task and at 15-min recovery.

2.5. Flow cytometry

The following PC populations were identified on the basis of cell surface protein expression using multi-parameter flow cytometry; total PCs, HSCs (Sutherland et al., 1994), early and late HSCs (Terstappen et al., 1991), and two EPC populations (Timmermans et al., 2009) (Fig. 2). In brief, 9 ml of ammonium chloride lysis solution (0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) was added to 1 ml of EDTA whole blood (10 min, RT). Phosphate-buffered saline (PBS, 5 ml) was added, samples were centrifuged (283×*g*, 7 min, RT), and the supernatants removed prior to incubation with monoclonal antibodies: CD34-FITC, CD133-PE, IgG Isotype-PE, CD3-PerCP, CD45-PerCP and CD8-APC-Cy7 (Becton–Dickinson, Oxford, UK), and CD38-PE-Cy7 (eBioscience, Insight Biotechnology Ltd, Middlesex, UK). Cell were washed, re-suspended in PBS/paraformaldehyde (1–2%), and stored in the dark (4 °C) until acquisition. Cells were read using a FACS-Canto II (Becton Dickinson, Oxfordshire UK). At least 1×10^6 gated lymphocytes and monocytes were acquired. Data were analyzed using Flowjo 7.4 (Treestar Inc, Ashland, OR, USA). Complete white blood cell counts were obtained using a Haematology analyser (Coulter ACT^{diff}, Beckman Coulter, High Wycombe, UK or Coulter GEN-S haematology analyser, Beckman-Coulter, Miami, USA). Numbers of PCs were then calculated using standard dual platform methods. Analyses was also adjusted for stress-induced changes in haemoconcentration as previously described (Bosch et al., 2005). Total PC, HSC, early HSC, late HSC, EPC and $CD8^+$ T lymphocyte numbers

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