The acute gravitational stress of parabolic flight affects red blood cell aggregation and functionality of circulating immune cells

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Introduction

Space exposome is the sum of biological, microbial, physical, and psychological stressors that influence crew members' physiological deconditioning in space. One of the biological systems that suffers a physiological deconditioning is the immune system. Briefly, this function is carried through circulating immune cells (neutrophils, monocytes, and lymphocytes) and organs (thymus, bone narrow, spleen, lymph node, and mucosa-associated lymphoid tissue).
The aim of this work was to assess if there are any relevant changes in the immune cells after a parabolic flight with a specific and unique profile. We hypothesize that the acute stress response caused by the intermittent exposure to hyper and microgravity could cause a change in the circulating leucocyte function and immunophenotype because of potential activation or de-regulation of some cell subtypes.

Materials and methods

Blood collection

Blood samples were obtained from 6 healthy human volunteers. From each sample, two aliquots were drawed; one stayed on ground whereas the other one was carried on a parabolic flight, being exposed to different gravitational loads. Both aliquots were maintained at equal temperature.

Parabolic flight

A parabolic flight campaign took place in Sabadell Airport (Barcelona, Spain) in March 2022 with 15 parabolas. This parabolic flight allowed for up to 8.5 seconds of microgravity per parabola, using a Mudry CAP10B aerobatic aircraft operated by Barcelona-Sabadell Aviation Club. This platform has successfully proven in the last decade to perform optimal parabolas with an average of 0.01g residual acceleration for both education and research purposes (Perez-Poch et al., 2016). Brief periods of hypergravity up to 3.2g preceded and followed each microgravity period. The samples were loosely attached to the cockpit avoiding vibrations or g-jitter, and the corresponding control samples were left on ground. Both samples were analysed in the Functional Cytomics Laboratory (IGTP) in the vicinities of the airport less than 2 hours after the flight.

Flow cytometry

Data were collected on an Attune™ NxT™ Flow Cytometer (Thermo Fisher), equipped with 4 lasers (405nm-violet, 488nm-blue, 561nm-yellow and 637nm-red), 14 fluorescent detectors and acoustic-assisted hydrodynamic focusing. Samples were prepared within 4-24h after parabolic flight for the assessment of red blood cells scattering, phagocytosis and oxidative burst, and for the analysis and quantification of T/B/NK-cells, according to the following protocols:

Red blood cells scattering: Briefly, 2µl blood were diluted in 1ml Hanks' Balanced Salts Solution (HBSS, Biowest). Diluted blood was acquired immediately and after a 15 minute-incubation at 37ºC by flow cytometry for red blood cell scattering assessment following a protocol described by Rico et al. (2018).

Phagocytosis and oxidative burst: Samples were prepared following a minimal perturbation protocol (Rico et al. 2021). 50µl blood diluted in 1ml HBSS were incubated with 10µl Hoechst 33342 (1mg/ml), 2µl Dihydrorhodamine 123 (0.01mg/ml) and 10µl pHrodo™ (1mg/ml) (Thermo Fisher). For stimulation, 1µl PMA (1mg/ml) was added. Non-stimulated control samples were prepared with 1µl DMSO. Samples were incubated for 30 minutes at 37ºC before flow cytometry analysis.

Immunophenotyping of TBNK cells: 100 µl blood were incubated with FITC-CD3, APC-CD4, Pacific Blue-CD8, Pacific Orange-CD16, AF700-CD19 and PE-CD56 (Sysmex). After incubation, samples were fixed and lysed with CyLyse™ (Sysmex) following manufacturer's instructions.

Data analysis: Data was analyzed using FlowJo™ v.10. For statistical analysis, GraphPad™ Prism v.9 was used.

Results

Red cell aggregation. Single cell analysis of erythrocytes was performed using flow cytometry. Single cell counts were as follows: median = 108951 cells, ranging from 83355 to 124822 cells (Group A) and median = 120506 cells ranging from 96252 to 140555 cells (Group B).

Oxidative burst. Single cell analysis of monocytes and neutrophils provided the following rhodamine 123 mean fluorescence intensity values: median = 36348 a.u. (arbitrary units), ranging from 23841 to 50336 a.u. (Group A) and

median = 32520 a.u. ranging from 22005 to 47900 a.u. (Group B).

Phagocytosis. Single cell analysis of phagocytes provided the following pHrodo mean fluorescence intensity values: median = 19902 a.u., ranging from 14850 to 25167 a.u. (Group A) and median = 22816 a.u. ranging from 18642 to 28794 a.u. (Group B).

Immunophenotyping. Peripheral blood obtained from 6 healthy donors was used for TBNK immunophenotyping. Flow cytometric results were analyzed using FlowJo™ v.10 in association with tSNE displays. CD3, CD4, CD8, CD19, CD56 and CD16 marker expression was studied before and after parabolic flight. Then, FCS files were concatenated and subjected to tSNE dimensionality reduction. $CD19^+$ B cells, $CD4^+$ Tcells and $CD8^+$ T cells, $CD4^+CD56^+$ NKTs, $CD56^+CD16^{dim}$ NKs, and $CD56^{dim}CD16^+$ NKs subsets were clearly identified. After dimensionality reduction, no differences were observed when comparing the two different conditions.

Discussion

We have applied two specific methods aimed at potential metabolic changes in leukocyte function, including oxidative burst and phagocytosis. In addition we have assessed the TBNK immunophenotyping, and all the experiments involved the use of acoustic focused flow cytometry. To do this, we compared two different groups of healthy subjects in the setting of a blind trial. Even non-significant results were obtained, our results show a different tendency when comparing the two groups. Regarding erythrocyte cell counting, we observed an increase in Group B when compared with Group A. Previous studies (Marijke et al., 2017) have shown the effects of microgravity on red cell aggregation, with impact in important differences of aggregated cells formed in space and on the ground. Based on the observed differences between two groups, we elucidated that the Group A was composed by the set of samples subjected to brief periods of hypergravity and microgravity when compared with blood control samples that were left on ground (Group B), showing less aggregated cells. Interestingly, oxidative burst measurements were increased in Group A, whereas phagocytosis was decreased in the same group. It has been described that the oxidative burst reaction depends on gravity, responding rapidly to gravity changes in just few seconds (Adrian et al. 2013). On the contrary, phagocytosis was also altered, suggesting that hypergravity and microgravity also modify phagocytic function in humans. Interestingly, we did not observe any significant change in cell counting and TBNK cell subsets, indicating that more prolonged dependence of time exposure to changes in gravity should be needed.

Conclusions

Taken together, our results suggest that short exposure to gravity variations may result in functional cellular changes that can be detected using flow cytometry. Cell immunophenotyping was unaffected, indicating that time exposure to gravity changes above a relevant threshold can promote further alterations. More studies will be needed to elucidate how gravity changes may alter cell immunophenotyping and function, with promise that these preliminary experiments provide valuable information after short exposure conditions.

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Ethics Statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. All participants had voluntarily given their written informed consent for this study.

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