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Changes in bioactive lipids signalling under Space conditions

Background. Microgravity that astronauts experience during missions has been involved in several cellular and molecular alterations, including those associated with the immune system and gastrointestinal (GI) trait [1]. Of note, the onset, progression, and outcome of inflammatory processes are regulated by specific endogenous lipids that act at the inflamed site to shape the magnitude of the immune response to avoid chronic inflammation [2]. Among these, two prominent groups of signalling lipids are represented by the specialized pro-resolving mediators (SPM) – a wide group of ω-3- and ω-6-derived compounds that act as main orchestrators of the resolution processes – and by endocannabinoids – the endogenous ligands of the receptors engaging the psychoactive compounds of Cannabis sativa and indica) [i.e. cannabinoid receptors 1 and 2 (CB1 and CB2)]. Even though these lipids have been consistently linked to immune and tissue homeostasis in virtually every known disease [2], their involvement in Space-related disorders has been largely neglected in Space biology [3]. Of note, our group has previously demonstrated that immune cells (e.g., lymphocytes) exposed to authentic microgravity – in the framework of the ROALD, RESLEM and SERiSM missions on board the International Space Station (ISS) – or to simulated weightlessness display altered metabolism and signalling pathways of the eCB system [4–6]. Yet again, to date, few studies – if any – have addressed the effect of microgravity on SPMs and the resolution system, nor its role on the lipid systems that control the GI homeostasis during Space travel. Thus, In the present study, simulated microgravity, achieved by means of the Rotary Cell Culture System (RCCS) developed by NASA, was used to assay the effect of weightlessness on the metabolism and signalling of SPMs and eCB system in human primary monocytes and on human GI cells, respectively.

Methods. PBMCs (peripheral blood mononuclear cells) were isolated from human blood samples and incubated at 1xg Earth gravity or 10-3xg RCCS-simulated microgravity for 24h [4]. Quantitative real-time PCR (qPCR) and polychromatic flow cytometry were performed to evaluate the gene and protein expression of SPM-related receptors and enzymes in PBMC-derived human primary monocytes, whereas production of bona fide SPM lipids was measured by liquid chromatography-mass spectrometry (LC-MS/MS). The activity of 5-LOX was assayed by means of commercially available fluorometric kits.

Human Caco-2 cells were chosen as a model of intestinal epithelial cells, as reported [7], and kept in adherence to Cytodex® microcarrier beads (microcarrier/cell ratio 1:20) [8] before being exposed to 1xg Earth gravity or 10-5 xg RCCS-simulated microgravity for 48h. Gene and protein expression of the eCB system enzymes and receptors were assayed by means of qPCR and Western Blotting.

Results. Human PBMCs that underwent 24h of simulated microgravity displayed an enhanced gene expression of pivotal SPM receptors such as GPR32, formyl peptide receptor 2 (FPR2, also known as ALX), GPR18 and Chemerin Receptor 23 (ChemR23), and of their biosynthetic enzyme 5-lipoxygenase (5-LOX) in respect to 1xg control samples. Furthermore, analysis of protein expression by polychromatic flow cytometry revealed that GPR32 and GPR18 underwent a significant up-regulation after 24h of microgravity. This effect was specific to CD14+ monocytes, but not to CD3+ lymphocytes. Microgravity also elicited a significant downregulation of 5-LOX in monocytes, a concomitant reduction of its activity, as well as it resulted in abated production of the prominent SPM resolving (Rv) D1 in LPS-stimulated cells.

On the other hand, Caco-2 cells exposed to RCCS-simulated microgravity displayed a significant rearrangement in the expression of eCB-related elements: in particular, 48h of weightlessness resulted in significantly reduced protein expression of CB1 and CB2 receptors and downregulation of the peroxisome proliferator-activated receptor γ (PPARγ) gene product.

Conclusions and future perspectives. More than 30 years have passed since the publication of the first papers – authored by Augusto Cogoli – that linked microgravity to immune cellular alterations. Since then, many other works have been published that characterized the molecular mechanisms underlying immune suppression processes observed in astronauts. However, very few of them addressed the role of bioactive lipids in these processes, with the vast majority investigating arachidonate-derived eicosanoids and, to a minor extent, eCBs [3], while other lipid congeners that have been pivotally involved in tissue homeostasis, (such as SPMs) have been barely studied to date.

Our data show, for the first time, that short exposure to microgravity significantly affects the signalling and metabolism of SPMs in monocytes, which are among the main orchestrators of the immune response and of its resolution [9]. SPMs and the cells that produce them play a major role in avoiding irreversible damage

that might arise from deviant inflammatory processes, and their impairment has been linked to virtually any pathological condition that features inflammation [2]. As a matter of fact, Space-related disorders display unresolved inflammation that might emerge from an impaired, altered or insufficient resolution system, suggesting a role of these lipids in microgravity-associated disorders. On the other hand, our preliminary data on Caco-2 cells indicate that microgravity does indeed affect eCB signalling even in the GI tract by affecting the gene and protein expression of crucial receptors of these lipid compounds.

SPM- or eCB-binding receptors and metabolic enzymes are currently investigated as clinical targets in many conditions [2,10,11], and this same strategy might well be exploited in the future to develop pharmacological countermeasures to either treat or prevent the disorders astronauts experience during missions.

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