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Simulated Microgravity Induces Apoptosis in Human Lymphocytes by a 5-Lipoxygenase Mediated Mechanism

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Introduction

Experiments performed during spaceflight clearly show that several cellular processes, such as oxidative metabolism, growth rates, signaling pathways and gene expression, are modified under conditions of weightlessness [1]. In particular, it has been suggested that reduced growth response in lymphocytes during spaceflight might be linked to apoptosis, based on morphological detection and cDNA microarray analysis [2] of space-flown human lymphoblastoid (Jurkat) cells. 5-Lipoxygenase (arachidonate:oxygen 5-oxidoreductase, EC 1.13.11.34; 5-LOX) plays a central role in interleukin-2 expression and activation of human lymphocytes, and is involved in the initiation of programmed death (apoptosis) triggered by unrelated stimuli in different human cells (reviewed in ref. 3). Remarkably, recent *in vitro* studies performed in the course of the 28th parabolic flight campaign of the European Space Agency have demonstrated that low gravity (~10^{-2}*g*) directly enhances the catalytic efficiency of a prototypal lipoxygenase, up to ~4-fold over the ground (1*g*) controls [4]. Therefore, we sought to simulate microgravity with the random positioning machine (RPM), also called three-dimensional clinostat, and with the rotary cell culture system (RCCS), in order to ascertain whether or not weightlessness might induce apoptosis in human lymphocytes. Also the possible involvement of the arachidonate cascade was investigated, as well as the role of mitochondria in the apoptotic program. Taken together, the data indicate that microgravity induces lymphocyte apoptosis and that 5-LOX is the "gravity sensor" which orchestrates this process.

Results

Simulated microgravity induces apoptosis in human lymphocytes. Exposure of human lymphocytes to μg led to a time-dependent apoptotic body formation, which was significant ($p < 0.01$) already at 24 hours (~3-fold over the control) and reached a level of 5- to 6-fold over the control 24 to 48 hours later. In the same time window we have previously observed a reduction of mitogenic activation of human lymphocytes exposed to microgravity [5], suggesting that μg-induced apoptosis might play a role in lymphocyte depression. On the other hand, lymphocytes incubated at 1*g* under the same experimental conditions did not show any significant sign of apoptosis.

Microgravity enhances 5-lipoxygenase, but not cyclooxygenase, activity in human lymphocytes. The possible role of the arachidonate cascade in lymphocyte apoptosis was ascertained by measuring 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) activity, which catalyze the committed steps in the biosynthesis of the most biologically active arachidonate derivatives, leukotrienes and prostanoids. The activity of 5-LOX in lymphocytes increased remarkably (~4-fold over 1*g* controls) already 2 hours after μg treatment, and it remained at least 3-fold higher than 1*g* controls during the following 70 hours. Unlike 5-LOX, COX activity did not change under μg conditions, suggesting that the lipoxygenase branch of the arachidonate cascade was upregulated in lymphocytes exposed to microgravity, whereas the cyclooxygenase branch was not.

The role of 5-LOX in microgravity-induced apoptosis was further investigated by treating lymphocytes with different enzyme inhibitors from the beginning of μg exposure and for up to 48 hours. The results obtained with the optimal concentration of each compound, as ascertained in pilot experiments, are summarized in Table 1. It shows that the 5-LOX inhibitors eicosatetraynoic acid (ETYA) and caffeic acid (CA) inhibited 5-LOX activity at 2 hours and apoptotic body formation at 48 hours, to similar extents (10-20% of the controls). On the other hand, the COX inhibitor indomethacin was ineffective (Table 1).

Microgravity induces mitochondrial uncoupling and cytochrome c release

Microgravity led to a time-dependent mitochondrial uncoupling, which was ~5.5-fold over the control 4 hours after exposure to μg and remained ≥ 4-fold over the controls in the following 68 hours. Conversely, ground (1*g*) controls did not show any significant mitochondrial uncoupling in the same time window. Treatment of lymphocytes with μg led to a ~5-fold increase in cytochrome c release after 8 hours, and cytochrome c release remained ≥ 4-fold higher than the 1*g* controls in the following 64 hours. The effect of μg at 8 hours

was prevented by ETYA or CA, but not by indomethacin, in a way which fully resembled their ability to inhibit 5-LOX activity (Table 1).

Compound	5-LOX activity (%)	Cytochrome c release (%)	Apoptotic bodies (%)
Vehicle	100	100	100
ETYA (10 μM)	10 ± 2*	20 ± 2*	20 ± 2*
CA (40 μM)	15 ± 2*	25 ± 2*	20 ± 2*
Indomethacin (10 μM)	100 ± 10	100 ± 10	100 ± 10

Table 1. Effect of various compounds on 5-lipoxygenase (5-LOX) activity, cytochrome c release and apoptotic body formation in human lymphocytes subjected to microgravity.

Values refer to measurements performed 2 hours (5-lipoxygenase activity), 8 hours (cytochrome c release) or 48 hours (apoptotic bodies) after exposure to microgravity, and are expressed as percentage of vehicle-treated cells (100% = 3850 ± 370 pmol.min^{-1}.mg protein^{-1} for 5-LOX activity, 1.250 ± 0.080 A_{405} units for cytochrome c release, and 22 ± 3% for apoptotic bodies). Treatment of human lymphocytes with any of the compounds listed, in the absence of microgravity, did not significantly affect cell death under the same experimental conditions. *Denotes $p < 0.01$ compared to vehicle-treated controls.

Discussion

Previous experiments from both flight and ground-based model systems clearly indicate unexpected alterations of human lymphocytes, leading to growth retardation and depression of mitogenic activation. However, the mechanism(s) by which microgravity inhibits lymphocyte proliferation remain(s) unknown, and do(es) not depend on the lack of binding of extracellular signaling molecules [5]. Here, we show for the first time that microgravity induces apoptosis of lymphocytes, and that the execution of the death program is based on a temporal chain of events, which start with the early (within 2 hours) activation of 5-LOX activity and continue with the 5-LOX-dependent damage of mitochondrial integrity (4 hours) and cytochrome c release (8 hours), leading to apoptosis (48 hours). Therefore, it can be suggested that 5-LOX is a "gravity sensor" which orchestrates the apoptotic events triggered by μg in human lymphocytes, most probably due to its ability to directly damage mitochondria.

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