EFFECT OF MICROGRAVITY ON THYROID CELLS

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ABSTRACT

We studied the effect of real microgravity on thyroid cells whose functionality influences cardiovascular, musculoskeletal and nervous system. At this end FRTL-5 thyroid cells were maintained during Texus 44 mission (February 7, 2008 from Kiruna, Sweden). When the parabolic flight brought the sounding rocket to an altitude of 264 km, the culture media without or with TSH were injected in the different samples and the weightlessness prevailed on board for 6 minutes and 19 seconds. Control experiments were performed in parallel on ground and in an on-board 1g centrifuge. Results showed that in the microgravity condition the cells did not present signs of cell proliferation, the sphingomyelinase moved to the nucleus probably influencing signal transduction, the cell membranes presented a rearrangement with modification of lipid composition of the culture medium and loss of the TSH-receptor. The modification of TSH-receptor could explain the absence of cell proliferation.

1. INTRODUCTION

Cell membranes are not randomly organized, but certain classes of lipids and proteins form microdomains [1]. The most studied class was constituted by cholesterol (CHO) and sphingomyelin (SM) enriched lipid rafts [2]. These microdomains are not artifacts [3] but constitute functional raft clustering at physiologically relevant temperatures [4]. They act as platforms for specific proteins [5] and regulate cell signalling [6]. A significant amount of thyrotropin receptor (TSH-R) reside within lipid rafts where its constitutive multimerization is regulated [7]. The CHO-SM relation is controlled by neutral-sphingomyelinase (N-SMase) activity [8]. In fact, the activation of N-SMase degrade SM producing ceramide and phosphocholine and freeing CHO [8]. It is important to notice that this enzyme is involved in the damage induced by radiation [9, 10] and the increase in ceramide pool is responsible for sensitivity to radiation [10]. We have recently demonstrated that ultraviolet C (UV-C) radiation-induced apoptosis of thyroid cells involved, together to other lipid enzymes, N-SMase activity [11]. The present study was performed in FRTL-5 cells, a strain of normal and differentiated rat follicular thyroid cells, obtained, characterized and patented more than twenty-eight years ago by Ambesi-Impiombato et al. [12]. These cells permanently express most in vivo tissue-specific thyroid characteristics, such as thyroglobulin synthesis and secretion, iodide active transport, peroxidase production and TSH sensitivity. FRTL-5 cells are induced to proliferate only when cultured in the presence of TSH [12]. TSH starvation induce virtually all cells to accumulate in G0/G1 cell cycle phase, blocking cell proliferation [13] and yielding cells more resistant to UV-C radiation-induced apoptosis [14]. Differences in proliferating and quiescent cell responses to UV-C radiations were associated with different modifications in lipid metabolism [11]. Curiously in our previous space mission the cells cultured with TSH did not proliferate and both their cell growth and lipid metabolism were similar to those of quiescent cells [15]. To establish if this effect could be due to the microgravity, the present study was performed during the Texus 44 mission.

2. MATERIALS AND METHODS

2.1. Cell cultures

Rat FRTL-5 cells were prepared and characterised in the Ambesi-Impiombato laboratory as previously reported [12]. Cells were grown in Coon's modified Ham F-12 medium additioned with 5% calf serum and 6 hormones: 10ng/ml glycil-l-histidyl-l-lysine acetate (Sigma), 10^{-8} M hydrocortisone (Sigma), 10 μg/ml insulin (Sigma), 10 μg/ml somatostatin (Sigma), 5 μg/ml transferrin (Sigma) and 10 mU/ml TSH (Sigma). FRTL5 were maintained at +37° C in 5% of CO_2, 95% humidity incubator. The cells cultured in the presence of TSH were stimulated to proliferate whereas those cultured without TSH remained in a quiescent state [12].

2.2. Texus 44 mission

Texus 44 was launched on February 7, 2008 at 11.30 (UT) from Kiruna, in Northern Sweden. The parabolic flight brought the sounding rocket to an altitude of 264 km. At this time the media without or with TSH were injected in different samples and the weightlessness prevailed on board for 6 minutes and 19 seconds. Just prior to the end of the microgravity period, the culture media were recovered and the cells were fixed with absolute ethanol. Control experiments were performed in parallel on ground and in an on board 1g centrifuge.

2.3. Morphological analysis

The cells were examined in OLYMPUS IX 51 light microscopy equipped with a OLYMPUS DP 50 camera system and analysed at 20x magnification.

2.4. Protein determinations

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Proteins of culture medium were determined as previously reported [8].

2.5. Lipid analysis

Lipids were extracted from culture medium with 20 volumes of chloroform/methanol (2:1 v/v). The organic phase was washed with 0.2 volumes of 0.5% NaCl as previously reported [8] The total amount of phospholipids was determined by measuring inorganic phosphorus [8]. The CHO was separated on thin-layer chromatography (TLC), using hexane/diethyl ether/formic acid, 80:20:2 by vol., as solvent. The phospholipids were separated on TLC by using chloroform:methanol: ammonia, 65:25:4 vol/vol, as solvent.

2.6. Western blot analysis

About 30 μg proteins of culture medium were submitted to SDS-PAGE electrophoresis in 10% polyacrylamide slab gel and transferred into nitrocellulose in 75 min as previously reported [16]. Membranes were blocked for 30 min with 0.5% no fat-dry milk in PBS, pH 7.5, and incubated over night at 4°C with anti-SMase, RNA polymerase II and TSH-R antibodies. Blots were treated with horseradish-conjugated secondary antibodies for 90 min. Visualization was performed with the enhanced chemiluminescence kit from Amersham. Immunoblot bands were quantified by Scion Image programme.

2.7. Immunofluorescence analysis

Immunofluorescence analysis was performed by using anti-SMase antibodies on the samples fixed in ethanol.

3. RESULTS

3.1. Cell growth delays in microgravity

The morphological analysis of FTRL-5 cells cultured in the presence or absence of TSH showed that in the control samples either in 1g centrifuge or on ground the cells with TSH presented signs of proliferation that were absent in the microgravity samples (Fig.1).

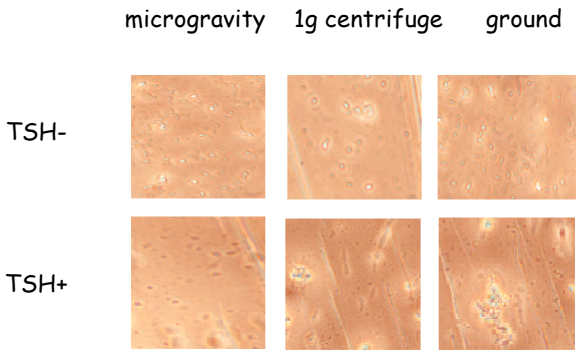

Figure 1. Morphological observation of FRTL-5 cells

3.2. Culture medium lipid composition

The analysis of lipid composition showed that in the control samples either in 1g centrifuge or in ground the phosphatidylethanolamine (PE) was higher whereas phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylinositol (PI), phosphatidylserine (PS) and CHO were lower in the culture medium in the presence of TSH (TSH^+, Fig.2 a,b) with respect to those without TSH (TSH^-, Fig.2 a,b). In microgravity condition, PE was similar in TSH^+ and TSH^- culture medium, PC, PI and PS had the behaviour similar to those of control samples, whereas SM and CHO were higher in TSH^+ culture medium with respect to TSH^- culture medium (Fig.2c)

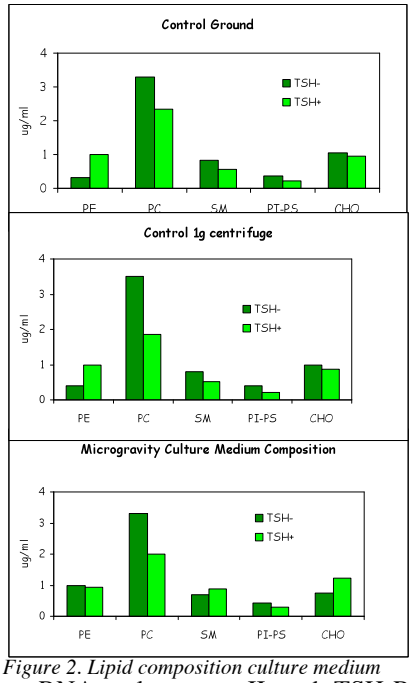

Figure 2. Lipid composition culture medium

3.3. SMase, RNA polymerase II and TSH-R in the culture medium

Immunoblotting analysis of culture medium showed that SMase did non change in the different samples and the RNA polymerase II was higher in microgravity condition either in TSH^+ or in TSH^- samples (Fig.3). The band of TSH-R was very strong in microgravity condition in the TSH^- sample (Fig.3) and the treatment with TSH limited the loss of TSH-R.

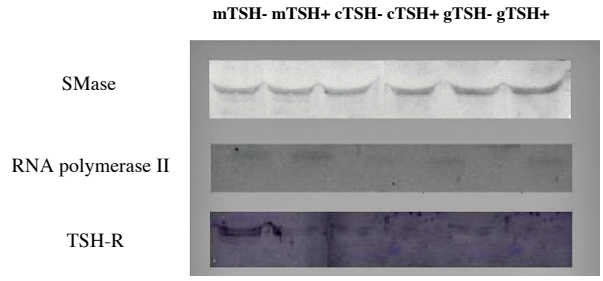

Figure 3. SMase, RNA polymerase II and TSH-R in the culture medium with TSH (TSH+) or without TSH (TSH-). m= microgravity; c=centrifuge; g=ground

3.4. Sphingomyelinase translocates to the nucleus

Immunofluorescence analysis showed that in microgravity condition the sphingomyelinase was strongly associated to the nucleus (Fig.4).

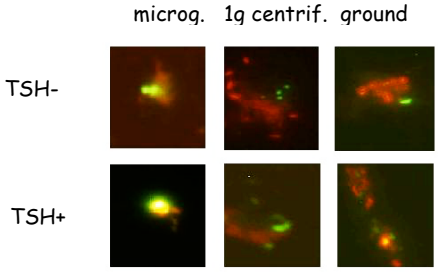

Figure 4. Sphingomyelinase localization (green=sphingomyelinase; red= nucleus)

4. DISCUSSION

Previous results in Eneide and Esperia mission demonstrated that FTRL-5 cell growth was delayed in space environment [15] but it was very difficult to establish if this effect in space was due to cosmic radiations or microgravity. This could be clarified with the reproduction in earth of cosmic radiations or microgravity separately. Unfortunately cosmic radiations are complex, being composed of galactic cosmic rays and solar particles [17] and at the moment an exact simulation of these radiations is not available on ground. In fact, today the simulation is usually performed by means of high energy protons or electrons from accelerators, gamma-rays from 60Co, UV light from a deuterium lamp, and soft X-rays or UV light from an electron synchrotron [18]. Regarding the simulation of the microgravity, Nelson and Jules reported that it is crucial to appreciate the distinction between the real microgravity environment and "weightlessness" or "simulated microgravity" [19]. The simulated microgravity i.e. by the clinostat reproduce only a part of microgravity biological effects [20] and the results sometimes appeared contradictory as occurs for the effect on testicular function [21]. It was demonstrated that simulated weightlessness changed the cytoskeleton of normal thyroid cells [22], increased the extracellular matrix proteins [23], reduced thyroglobulin, ft3 and ft4 secretion [24] and induced programmed cell death [25, 26] of thyroid carcinoma cells. No data exist about the effect of real cosmic radiation or microgravity on thyroid normal cells. Our data demonstrated for the first time that the effect of real microgravity on FRTL-5 cells reduces cell proliferation, induces a cytoplasm-nucleus translocation of sphingomyelinase and changes the content of SM, CHO and TSH-R in the culture medium. It is possible to hypothesize that real microgravity induces either a intracellular molecular translocation, by influencing signal transduction or a rearrangement of specific sections of cell membrane i.e. lipid rafts rich in SM and CHO content, which act as platform for molecular receptors. The modification of TSH-R could explain the absence of cell growth in the samples cultured in the presence of TSH.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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