MICROTUBULE DISRUPTIONS AND REPAIR PHENOMENA IN CULTURED GLIAL CELLS UNDER MICROGRAVITY.

Maria Angela Masini¹, Felice Strollo², Franco Ricci³, Martina Pastorino¹, Bianca Maria Uva¹,
¹Dipartimento di Biologia, Università di Genova, Italy, ²Unità Operativa Endocrinologia e Malattie del Ricambio INRCA & Università di Roma La Sapienza Italy, ³ENEA C.R. Casaccia - Roma Italy.

The structure of animal cell cytoplasm is based upon a scaffolding of proteins called cytoskeleton that is essential for many processes ensuring mechanical support, movement, adhesion, polarity and intracellular trafficking. The cytoskeleton is composed by filamentous proteins including microfilaments (actin), intermediate filaments (differing from cell to cell type) and microtubules (tubulins) all joined by cross-linking proteins. Microtubules are responsible for mitotic spindle assembly and chromosome movements as well as vesicular and cytoplasmic organelles movements inside single cells. Gravitational vector changes cause severe damages to the cytoskeleton, as observed in lymphocytes during space flight (Cogoli-Greuter et al., 1994; Lewis et al., 1998) and in ground-based experiments (Uva et al., 2002a,b,c).

The aim of the present study was to assess whether addition of minerals to the culture medium might repair or prevent microtubules alterations in low g. In this experiment C₆ cells (from rat astroglioma) were submitted to 3D RPM rotation (56 Deg/sec; 10⁻⁶ g) for 1h with addition to the culture medium (D-MEM) of: 1) mineral salt integrators (potassium and magnesium citrate, 10mg/ml), 2) calcium (5 µg/ml) and 3) magnesium (0.70 mg/ml). Control cells were cultured in D-MEM without any added minerals whatsoever and kept rotating for the same time. Static controls (1xg) were treated in parallel and positioned on the supporting frame of the RPM in order to have their cells facing the same vibration stress as in modeled microgravity samples. At the end of the rotation, the cultured cells were fixed (4% paraformaldehyde in PBS) and submitted to immunohistochemistry using an antibody to α-tubulin. Apoptosis was visualized by immunostaining of an apoptosis-related protein (caspase 7 executioner) and analysis of DNA fragmentation which was visualized by TUNEL method (Terminal dUTP Nick End Labeling). Nuclei were stained with 4,6-diammino-2-phenylindole-dyhydro chloride (DAPI). The cells were observed by conventional epifluorescence microscope (Olympus). Percent cells with well organized microtubular array, cells in mitosis and caspase positive cells, cultured in control and modified media, were counted from 3 randomly chosen fields in 5 slide preparations per sample. Statistical analyses made use of ANOVA with a confidence of 95%. Data were expressed as means ± SD.

In 1xg control the microtubular array was well organized (Fig. 1a), microtubules radiated from the organising centre to reach the plasma membrane. After 1h at RPM rotation in the cells cultured in D-MEM, without mineral salts or metal addition, microtubules appeared to be highly disorganized and shortened (Fig. 1b). Conversely, in cells cultured in D-MEM enriched with mineral integrators (Fig. 1c), calcium (Figs. 2a, b) or magnesium (Figs. 2c, d), the microtubular array remained well organized (Fig. 3).

Figure 1 A, B, C. α-tubulin immunohistochemistry. a) static 1xg control: microtubules are well organized, b) after 1h in modeled microgravity: microtubules appear to be highly disorganized. c) after 1h at modeled microgravity in the cells cultured in D-MEM enriched with minerals integrators, the microtubules appear to be well organized. 1800x.

Figure 2 A, B, C, D. α-tubulin immunohistochemistry. a, b: D-MEM with addition of calcium: a) static 1xg control, b) modeled microgravity. c, d: D-MEM with addition of magnesium: c) static 1xg control, d) 1h modeled microgravity. The microtubules remain well organized in both conditions. 800x.
The number of apoptotic cells was very high in the cultures containing only D-MEM, but got back to control levels (11±4.2% versus 70.62±11.06%) after magnesium addition, while the number of cell division increased both with calcium and magnesium (Fig.4A, B).

![Figure 4 A, B. Number of cell divisions (A), and number of apoptic cells (B) after 1 h at low g in controls and in D-MEM modified by addition of calcium and magnesium. Means ±SD](image)

Our data confirm previously reported observations that severe microtubular changes occur in low g and there is a lack of microtubular self organization under modeled or real microgravity conditions (Papaseit et al.; 2000; Tabony, 1994). However divalent ions, as calcium and magnesium, are known to play a major role in the regulation of microtubular polymerization in vivo under earth gravitational force. In fact a high magnesium concentration is needed to assemble tubulin dimers into microtubules in vitro (Gaskin, 1981).

The present results show that the addition of calcium or magnesium to the culture medium results in protection of microtubular integrity and chromatin, preventing apoptosis and enhancing cell divisions, probably due to the pivotal role of divalent ions in building the mitotic spindle and the cleavage furrow.

In conclusion these data show that an enriched metal concentration in the extracellular compartments may prevent low g alterations at the cellular level.

REFERENCES


