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HYPERGRAVITY EFFECTS ON PROLIFERATION AND DIFFERENTIATION OF C2C12 MUSCLE-LIKE CELLS

Abstract

Purpose This study aimed at investigating muscle-like cell behaviour under altered gravity conditions. C2C12 cells underwent different hypergravity intensity stimulations in the Large Diameter Centrifuge facility of ESTEC (through the ESA Education Spin Your Thesis! programme). Proliferation and differentiation were compared to control cultures carried out at earth gravity force. Methodology Both proliferating (15,000 cells/cm2) and differentiating (85,000 cells/cm2) cultures were exposed for 2 h at different hypergravity intensities (5, 10 and 20 g). Thereafter, cells have been immediately fixed for cytoskeleton conformation analysis or cultured for further 24 h and then fixed or frozen to perform appropriate assays. Both ds-DNA (PicoGreen assay) and total protein content (bicinchoninic acid method) assessments were carried out in cascade on the same samples. The organization of actin microfilament network in treated and non-treated cells has been evaluated by F-actin staining with TRITC-phalloidin, according to standard protocols. A similar procedure was followed for immunostaining of myogenin and myosin. All experiments were carried out in triplicate. Results Hypergravity positively affects proliferation: cultures that underwent 10 and 20 g stimuli exhibited a significant increment of DNA content with respect to the 1 g and 5 g experiment ($p < 0.05$). Protein synthesis capability showed similar results. F-actin filament thickness increments at higher g values, compatible with a rearrangement already described in the literature. Myogenin-positive nuclei was 10This conflict could be explained with an enhancement of the differentiation process following the hypergravity treatment: the expression of myogenin, an intermediate differentiation marker, is reduced because of an acceleration of the differentiation resulted in a precocious expression of late markers like myosin, as confirmed by the well sustained formation of myotubes at higher g values. Conclusion Proliferation and metabolism of C2C12 cells were positively affected by hypergravity: both the DNA and the protein levels are duplicated in 20 g cultures respect to the control cultures. Furthermore, the differentiation process is faster and better sustained at higher g level, as demonstrated by a down-regulation of myogenin followed by a myosin up-regulation.