Motor Coordination in Space: An Analysis of the Effects of Microgravity on XRCCI DNA Repair Protein Function

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Method Article

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Abstract

While in space, astronauts have been known to face exposure to stressors that may increase susceptibility to DNA damage. If DNA repair proteins are defective or nonexistent, DNA mutations may accumulate, causing increasingly abnormal function as one ages [1]. The DNA single-strand break repair protein XRCC1 is important for cerebellar neurogenesis and interneuron development [2]. According to previous studies, a deficiency of XRCC1 can lead to an increase in DNA damage, in mature neurons, and ataxia (a progressive loss of motor coordination) [2]. I propose to address how XRCC1’s efficiency can change in microgravity conditions. This experiment’s relevance is underscored by the importance of motor coordination and physical fitness for astronauts; determining the potential effects of microgravity on XRCC1 is crucial for future space exploration.

Introduction

Reagents

Equipment

Procedure

1. Immature mammalian cerebellar interneurons should be cultured with a sufficient nutrient medium and maintained at 23°C.

2. Experimental Design: The interneurons will be split into four groups: those exposed to 1) UV radiation and microgravity on the ISS, 2) UV radiation and no microgravity on Earth, 3) no UV radiation and only microgravity on the ISS, and 4) neither UV radiation nor microgravity on Earth (control). Each group will consist of four samples. To compensate for UV irradiation on the ISS, I plan to measure the magnitude of this (in J/m²) and replicate it on all Earth samples. Sample Ct’s (threshold number of cycles), average (avg) Ct’s for each condition, ΔCt (avg Ct’s of each condition compared to control condition), ΔΔCt (difference between each condition’s ΔCt and control ΔCt), and 2-ΔΔCt (fold relationship) values, as well as null hypothesis t-tests (p-value threshold of 0.05), will be calculated and conducted to assess statistical significance of results. Higher Ct’s correspond to lower initial DNA concentrations.

3. An International Space Station (ISS) laboratory is required. Two ultraviolet (UV) light bulbs (40W) will be placed in the ISS, and two on the Earth.

4. After exposure to their respective conditions (see Experimental Design #2) for ~72 hours, the interneurons will be centrifuged, disrupted (with Buffer RLT Plus), homogenized (via Bead Mill), and lysed and purified (via the DNeasy Plus Mini Kit; handbook by Qiagen will be closely followed to maintain adherence to recommended protocols).
5. NanoDrop 2000 will assess the quality and quantity of the extracted DNA; if needed, the DNA samples will be exposed to RNase and protease for further purification.

6. The MAP2 (microtubule associated protein 2) gene will be amplified with PCR (via Taq polymerase); MAP2 is important for microtubule assembly in neurogenesis and its PCR-primer-binding sequences (forward primer: CCACCTGAGATTAAGGATCA; reverse primer: GGCTTACTTTGCTTCTCTGA) contain many C-T sequences that are especially prone to damage from UV radiation [3,4,5].

7. The iTaq™ Universal SYBR® Green One-Step Kit will be used for quantification of DNA products.

References


