Examining the effect of Cumulative Low Dose Radiation on TK6 Human Lymphoblastoid Cells During Simulated Microgravity

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Introduction
Background

- Many researchers study the effect of microgravity and space irradiation separately.

- Most space radiation research is currently focused on a single high dose of radiation.

- The effect of cumulative low-dose irradiation on cell damage is not well known.
Objectives of Internship

Our goal was to assess the combined effect of continuous low-dose radiation during simulated microgravity conditions.
Mechanism of Radiation Damage
Introductory Terminology: Absorbed Dose

Gray = the SI unit of absorbed dose
   = 1 Joule of radiation absorbed / Kilogram

Rad = (Radiation Absorbed Dose) the old unit of absorbed dose
   = 0.01 Joule of radiation absorbed / Kilogram

One Gray = 100 Rads
Introductory Terminology: Equivalent Dose

- **Sievert**: Is a measure of equivalent / effective dose.

  Each tissue is assigned a weighting factor
  
  \[ \text{Sievert} = \text{Gray} \times \text{weighting factor} \]

- **Rem**: (roentgen equivalent in man) the old unit of equivalent or effective dose
  
  \[ \text{Rem} = \text{Rad} \times \text{weighting factor} \]

  One Sievert = 100 REM
# Radiation Weighting Factor

<table>
<thead>
<tr>
<th>Type and Energy Range</th>
<th>Radiation Weighting Factor, $W_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photons</td>
<td>1</td>
</tr>
<tr>
<td>Electrons</td>
<td>1</td>
</tr>
<tr>
<td>Protons</td>
<td>2</td>
</tr>
<tr>
<td>$\alpha$-Particles, fission fragments, heavy nuclei</td>
<td>20</td>
</tr>
</tbody>
</table>

A continuous curve is recommended with a maximum of 20 for the most effective neutrons of about 1 MeV.
Tissue Weighting Factor

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$W_T$</th>
<th>$\sum W_T$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow, breast, colon, lung, stomach</td>
<td>0.12</td>
<td>0.60</td>
</tr>
<tr>
<td>Bladder, esophagus, gonads, liver, thyroid</td>
<td>0.05</td>
<td>0.25</td>
</tr>
<tr>
<td>Bone surface, brain, kidneys, salivary glands, skin</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Remainder tissues$^a$</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Equivalent Dose

\[ \text{Equivalent Dose} = \sum \text{Absorbed dose} \times W_R \times W_T \]
Materials and Methods

- **TK6 Human Lymphoblastoid cells**
  
  Sensitive to radiation
  
- **Hardware**
  
  Low Dose Gamma Ray Source (Cesium - 137)
  
  High Aspect Ratio Vessel (HARV)
  
  Rotary Cell Culture System (RCCS)
  
- **Experiment Design**
  
  Experiments 1-5, 9: 40,000 cells/mL for 3 days
  
  Experiments 6a: 80,000 cells/mL for 2 days
  
  Experiments 6b, 7, 8: 80,000 cells/mL for 3 days
Setup

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# Results

- 9 Experiments were conducted
- Data was gathered from 5

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Power surge caused radiation to stop</td>
</tr>
<tr>
<td>2</td>
<td>Usable</td>
</tr>
<tr>
<td>3</td>
<td>Usable</td>
</tr>
<tr>
<td>4</td>
<td>Usable</td>
</tr>
<tr>
<td>5</td>
<td>Useable</td>
</tr>
<tr>
<td>6a</td>
<td>Useable</td>
</tr>
<tr>
<td>6b</td>
<td>Rotation device stopped working</td>
</tr>
<tr>
<td>7</td>
<td>Failure to proliferate</td>
</tr>
<tr>
<td>8</td>
<td>Failure to proliferate</td>
</tr>
<tr>
<td>9</td>
<td>Harvested for future RNA analysis</td>
</tr>
</tbody>
</table>
Results

Average increase in Rotation Only group vs Control = $1.55 \pm 0.13$
Results

Average percent decrease due to Radiation vs Control = 14.01% 6.59%
Results

- Predict the expected cell numbers for the combined effect of rotation & radiation based upon previous data

- Example: Experiment 2
  
  Control = **502,750 cells/mL**
  
  \[ \times 1.13 \text{ (rotation growth factor observed compared to control)} \]
  
  = **568,300 cells/mL**

Reduce 568,300 by 38.24% (radiation damage observed compared to control)

= **350,982 cells/mL**

(Expected effect of combining rotation & radiation together)
Results

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Expected (cells/mL)</th>
<th>Observed (cells / mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>350,982</td>
<td>256,550</td>
</tr>
<tr>
<td>3</td>
<td>1,838,223</td>
<td>670,200</td>
</tr>
<tr>
<td>4</td>
<td>195,553</td>
<td>194,650</td>
</tr>
<tr>
<td>5</td>
<td>1,169,681</td>
<td>841,850</td>
</tr>
<tr>
<td>6a</td>
<td>610,403</td>
<td>460,975</td>
</tr>
</tbody>
</table>

An Average of 28.68 ± 10.08% Lower than Expected
Results

Rotation & Radiation
Expected vs. Observed

<table>
<thead>
<tr>
<th>Exp</th>
<th>Expected</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>350982</td>
<td>256550</td>
</tr>
<tr>
<td>3</td>
<td>1838223</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>670200</td>
<td>195553</td>
</tr>
<tr>
<td>5</td>
<td>1169681</td>
<td>194650</td>
</tr>
<tr>
<td>6 (2day)</td>
<td>610403</td>
<td>460975</td>
</tr>
</tbody>
</table>

Cells/mL
Discussion

The data suggests a **synergistic effect** exists when Rotation (simulated microgravity) and Radiation are combined together.

Current models for determining upper limit radiation doses are based upon terrestrial models.

Weighing factors:

\[
\text{Equivalent Dose} = \sum \text{Absorbed dose} \times W_R \times W_T
\]
Discussion

Perhaps there is a heretofore undiscovered “third weighting factor,” a so-called “gravity weighting factor: $W_G$.”

Equivalent Dose = $\Sigma$ Absorbed dose x $W_R$ x $W_T$ x $W_G$
Discussion: Caveats

1. The cell numbers differed substantially from experiment to experiment

2. A rotating bioreactor can only incompletely approximate microgravity

3. Cells may have been dying due to other reasons
   • E.G. Stress from presumably being packed together?
   • Currently testing for oxidative stress markers (GSH, LPO)

4. Radiation damage was not completely assessed via micronuclei analysis (currently underway)
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