Testing Sodium Butyrate Anti-Cancer Properties on K562 Cells

By: Krystal Alston, Jasmin Bates, Ratita Dennis, and Cynthia Vu
Background: Cancer

Cancer – A genetic disease caused by an uncontrolled division of abnormal cells
- Benign tumors vs. malignant tumors

Leukemia – A cancer of white blood cells
- Acute vs. chronic leukemia
- 4 types of leukemia: CML, CLL, ALL, AML
- Increase in % of WBC

Video:
http://www.youtube.com/watch?v=WNA2XakMFW0
Human Blood Smear

Normal Blood Sample

Leukemia Blood Sample
K562 Cell Line

Cell lines have mutations that make them immortal and allow the cells to divide continuously which is important for research purposes.

A cell line from a 53 year old woman with chronic myelogenous leukemia (CML)

Non-adherent cells

Highly undifferentiated blood cells
Hematopoiesis

Blood stem cell

Myeloid stem cell

Myeloblast

Granulocytes
- Eosinophil
- Neutrophil
- Basophil

B lymphocyte

T lymphocyte

Natural killer cell

Lymphoblast

Lymphoid stem cell

Red blood cells

Platelets

White blood cells
Sodium Butyrate

- Naturally occurring compound
- Short chain fatty acid
- Inhibits cell growth (cell division)
- Induces blood cell differentiation
- Alters gene expression
Purpose & Hypothesis

**Purpose**- to test anti-cancer properties of Sodium Butyrate
Forcing K562 cells to mature would stop the uncontrolled growth

**Hypothesis**- Sodium Butyrate will slow the growth and induce Red Blood Cells formation (Mature)
Overview of Experimental Design

Starting Day
- Seed K562 cells
- treat w/ 1mM, 5mM, 20mM of Sodium Butyrate
- 4 replicates/each treatment

Day 1 & 2
- Count cells
- % dead w/ trypan blue

Day 3
- Count cells
- % dead w/ trypan blue
- Benzidine Assay (RBC)
Experimental Design

**Independent Variable** - Sodium Butyrate treatment

**Dependent Variable** -
1) growth of K562 cells
2) Death of K562 cells
3) production of RBCs

**Control** - Untreated K562 Cells

**Control Variables** - density of K562 cells; amount of time of growth; temperature; light and carbon dioxide
Aseptic Technique & Cell Culture

Sterile Conditions

Laminar Flow Hood
- Protects cells and ‘work area’ from contamination

Cell Culture
- Flask with cell growth medium

Incubator
- 37°C and 5% CO₂
Measuring Cell Growth and Death: Hemacytometer

Trypan Blue?
Results: Cell Growth

The graph shows the average cell number per grid over three days for different treatments: untreated, 1mM, 5mM, and 20mM. The untreated group shows a significant increase in cell number over time, while the other treatments show less pronounced growth, with the 20mM group maintaining a relatively constant cell number.
Result: Percent Cell Death

Average Percent of Cells Dead

Day

Average Percent Cells dead (per grid)

untreated
1 mM
5 mM
20 mM
Benzidine Assay

- Indication of hemoglobin presence
- Redox reaction
- Measure absorbance with plate reader

Benzidine (colorless) + Hemoglobin + H₂O₂ → Oxidized Benzidine (Blue)
Lose e⁻ to hemoglobin
Result: Hemoglobin Levels

Hemoglobin Levels

Absorbance (580nm)

<table>
<thead>
<tr>
<th>Treatment Concentration</th>
<th>0.35</th>
<th>0.30</th>
<th>0.25</th>
<th>0.20</th>
<th>0.15</th>
<th>0.10</th>
<th>0.05</th>
<th>0.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.15</td>
<td>0.20</td>
<td>0.25</td>
<td>0.30</td>
<td>0.35</td>
<td>0.30</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>1mM</td>
<td>0.10</td>
<td>0.15</td>
<td>0.20</td>
<td>0.25</td>
<td>0.30</td>
<td>0.35</td>
<td>0.30</td>
<td>0.25</td>
</tr>
<tr>
<td>5mM</td>
<td>0.15</td>
<td>0.20</td>
<td>0.25</td>
<td>0.30</td>
<td>0.35</td>
<td>0.30</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>20mM</td>
<td>0.35</td>
<td>0.30</td>
<td>0.25</td>
<td>0.20</td>
<td>0.15</td>
<td>0.10</td>
<td>0.05</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Gel Electrophoresis

**Purpose:** Introduce method of analyzing cancer at the level of DNA

- Agarose gel in 1xTBE buffer
- Separate DNA based on size
- Cut and uncut samples
  - Cut samples travel further
  - Larger quantities produce thicker bands
Gel Electrophoresis Result

1.0 mg cut  0.5 mg cut  uncut
Conclusions

• The data supported the hypothesis.

• Cell growth of K562 cells was inhibited in a dose dependent way.

• Cell death increased in a dose dependent manner.

• Differentiation of K562 cells into RBCs occurred at the higher concentration.
  • Assay may not have been optimized/sensitive to the number of cells.
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thank you!