Using ELISA to Quantify β-Catenin Levels by GSK-3β Inhibition in HEK293T Cells

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Background

- Bipolar disorder
  - Recurrence of mania and depression cycles
  - Affecting 2% of the world population\(^1\)
  - Pathogenesis not well understood\(^2\)
Background

- Lithium (Li⁺)
  - First-line treatment for bipolar patients\(^2\)
  - Less than half of patients respond to treatment\(^2\)
  - Unclear how lithium exerts its clinical efficacy
Background

**TCF/LEF Response**

<table>
<thead>
<tr>
<th></th>
<th>Relative TCF/LEF Reporter Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIRON99021</td>
<td>HEK293</td>
</tr>
<tr>
<td>LiCl</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
</tr>
</tbody>
</table>

**Diagram:**

- **CHIRON99021- GSK-3β Inhibitor**
- **Lithium**
- **GSK-3b Inhibitor**
- **Inhibition**
- **Accumulation**
- **β-Catenin**
- **Activation**
- **Proposed Pathway for Mood Stabilization in Mouse Models**

**FireFly Lucif. reporter**
Purpose

- Li$^+$ and CHIRON99021
  - If the accumulation of the β-catenin causing the activation of TCF/LEF reporter
  - The dose- and time-dependent effects on levels of β-catenin
- Characterize novel GSK-3β inhibitors
  - TCF/LEF transcriptional activation
  - B-catenin stabilization
Methods

- **Cell Culture**
  - HEK293T-pBARLS cells

- **Total Protein Measurements**
  - Lowry Assay

- **B-Catenin Detection**
  - Sandwich Enzyme-linked Immuno-absorbent Assay (ELISA)
Over All Doses Tested, Lithium Shows the Greatest Effect on Total Levels of β-Catenin After 24hrs
Of All the Concentrations Collected, 10mM has Generated the Highest β-Catenin Levels
CHIRON99021 dramatically increased β-Catenin Levels as early as 8hrs
A Clear Correlation Exists between Treatment Concentrations and $\beta$-Catenin Response After 8hrs
At 25uM Novel Compound BRD-K98958073 Significantly Increases β-Catenin Levels Even Compared to CHIRON99021
Novel GSK-3β Inhibitor

BRD-K98958073-001-04-1
Summary

- Lithium shows a higher accumulation of β-catenin after 24 hours
- CHIRON99021 shows its higher accumulation of β-catenin as early as 8 hours
Summary

- CHIRON99021 dose-dependently increases β-catenin levels more consistently than Li⁺.
  - The mechanism by which Li⁺ activates TCF/LEF transcription may differ from that of CHIRON99021.
Summary

- BRD-K98958073**
  - Most effective novel inhibitor of GSK-3β assayed
  - ATP Competitive
Future Applications

- Understanding how Li$^+$ activates TCF/LEF transcription
  - Potential new biological targets for bipolar therapeutics.
- More effective mood stabilizers/GSK-3β inhibitors.
Acknowledgements

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References

(1) Bipolar disorder in adults. National Institute of Mental Health booklet; 2010 Aug 31
