

Eltrombopag, a Thrombopoietin Agonist, Causes Neuronal Iron Deficiency and Impaired Dendrite Branching During Development

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Introduction

Iron deficiency (ID)

- One of the most prevalent nutrient deficiencies
- Affects about 2 billion people world wide
- Affects 40-50% of pregnant women
- ID negatively effects early-life brain development and cognitive function

Thrombocytopenia

- Hematological condition affecting 70% of preterm infants, characterized by low platelet counts
- Platelet transfusion is standard treatment but also associated with increased risk of infection and immune dysfunction side effects
- Thrombocytopenic neonates could benefit from thrombopoietin mimetic therapy (e.g. Eltrombopag, ELT)

Hypothesis

Eltrombopag may chelate iron and cause ID, which could impair neuronal structural development

Results

ELT Decreases Dendritic Arbor Complexity

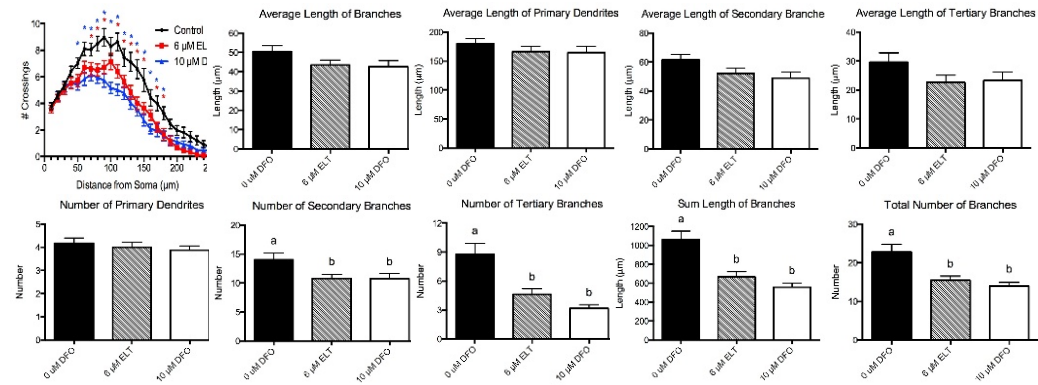


Figure 3: Eltrombopag treatment significantly decreases the number and length of dendrite branches. Data are presented as mean ± SEM. Groups with different letter superscripts are different by one-way ANOVA and Tukey's post-hoc test.

Summary & Future Directions

Conclusions

- ELT increased *TfR1* mRNA levels to a similar magnitude as DFO, indicating ELT causes neuronal ID.
- ELT treatment led to lower *BdnfVI*, *Cam2a*, and *Vamp1* mRNA levels, indicating impaired neurodevelopment and synaptic function and plasticity.
- ELT caused an overall reduction in hippocampal neuron dendritic arbor complexity, with decreased branch number and length but no change in primary dendrite number or length.
- Overall our data suggest that treating thrombocytopenic neonates with ELT may impair brain development

Future Directions

- Is there a level of Eltrombopag treatment that could be safe to use for neonates?
- Does Eltrombopag cross the blood-brain-barrier?
- Thrombocytopenia will be induced in pregnant female mice and Eltrombopag treatment will be used. Hippocampal neurons will be harvested from offspring and assessed for impairments in neuron development.
- Neuron density measurements will be conducted to assess if Eltrombopag negatively impacts neuron viability.

Experimental Design and Methods

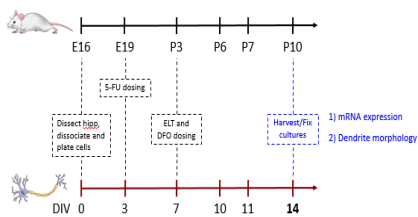


Figure 1. Experimental Design Summary. Hippocampal cells were harvested from embryonic day 16 mice. Neurons were grown on coverslips and treated with either 6 μM ELT, 10 μM DFO or no treatment from 7 days *in vitro* (DIV) to 14DIV. mRNA expression and dendrite morphology were assessed.

Figure 2: Dendrite Tracing

Immunocytochemistry was performed for MAP2 (red) and hrGFP (green). Primary (cyan), secondary (blue), and tertiary (yellow) dendrites were manually traced. Number and length of dendrites and branches were calculated. Sholl analysis was performed for overall dendrite complexity

ELT induces ID and impairs expression of neurodevelopment genes

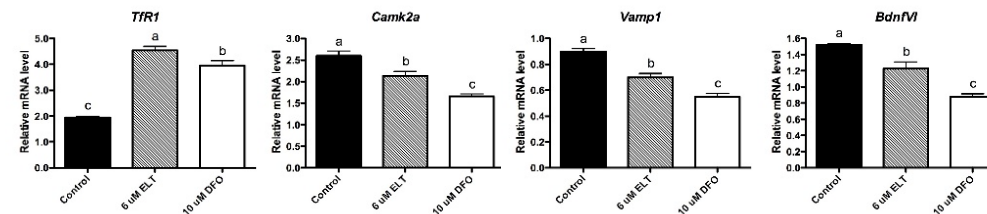


Figure 4: Eltrombopag treatment increases *TfR1* gene expression and decreases expression of *BdnfVI*, *Camk2a*, and *Vamp1*. qPCR mRNA expression analysis for genes indexing neuronal iron status [i.e., *TfR1* (iron transporter gene)] and dendrite and synapse development [i.e., *Camk2a*, *Vamp1* and *BdnfVI*]. Data are presented as mean ± SEM. Groups with different letter superscripts are different by one-way ANOVA and Tukey's post-hoc test.

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