Identifying Early Developmental Neurotoxicity Modeled in a Cerebral Organoid System

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Introduction

Lack of Clinical Data Leads to Fetal Neurodevelopmental Risks

- Pregnant individuals are typically excluded from traditional clinical drug trials resulting in a lack of clinical data which complicates medical decisions regarding the balance of maternal health and prenatal neurological development
- Retrospective computational methods have identified potential neurotoxic drugs[1]
- To expand knowledge of drug neurotoxicity effects in fetal populations alternative screening methods are essential
- Cerebral organoids provide an alternative model system for screening drug neurotoxicity

Organoid Workflow

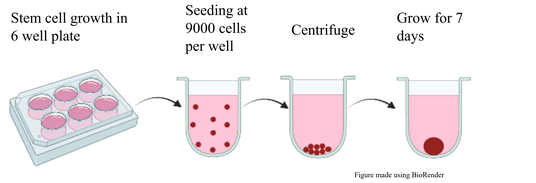


Figure 2. The process of forming a cerebral organoid begins with the culture of induced pluripotent stem cells. The organoid is then formed using centrifugation followed by differentiation using dual SMAD inhibition. GelCAD (a custom biomaterial) was used to encapsulate the organoid after 10 days promoting homogeneity and polarizing neuron maturation.

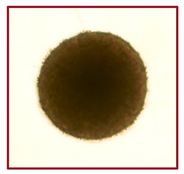


Figure 1. Cerebral organoid in solution after 7 days of growth

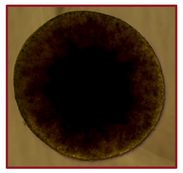


Figure 3. Cerebral organoid embedded in GelCAD after 10 days of growth

Viability & Growth Assays

Size and Shape Characterization

Organoid Dosing Procedures

- Organoids were dosed with folic acid, valproic acid, or gabapentin starting on day 20
- Pharmaceutics were administered on days 20 and 22
- Concentration was administered on a logarithmic scale
- After 30 days, organoids were fixed and stained with SOX2 and βIIIT

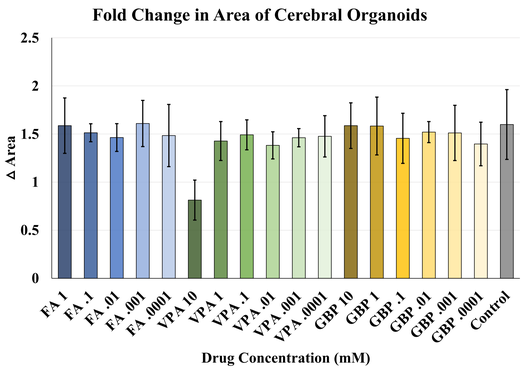


Figure 4. Fold change in organoid area was measured with the initial measurement on day 20 and the final measurement on day 30. Organoids under a circularity measurement of 0.7 on day 20 were excluded due to organoid viability concerns. Data indicated neurotoxicity occurred in 10 mM valproic acid dosed organoids.

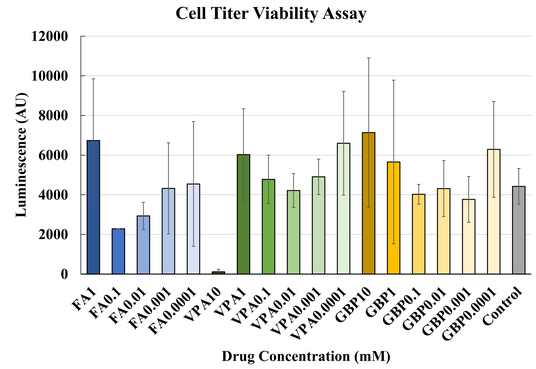
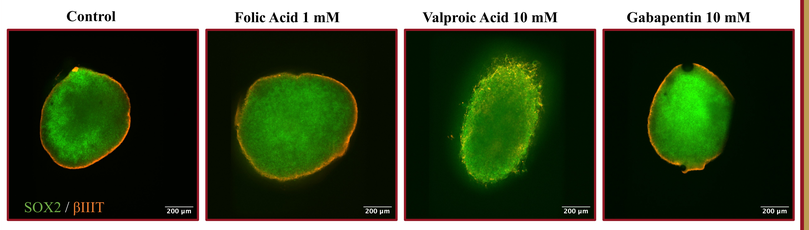


Figure 5. Cell titer assay was performed following the Cell-Titer Glo Luminescent Cell Viability Assay protocol. Results indicated viability in all cells except for those with valproic acid at a concentration of 10 mM.

Fluorescence Imaging

High Contrast



Identical Brightness

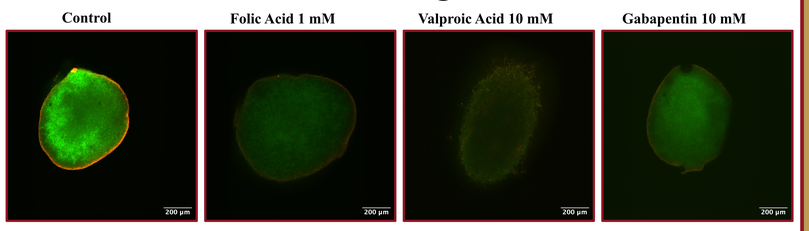


Figure 6. Day 30 organoids immunostained with SOX2 and βIIIT, visualized by spinning disk microscopy. Scale bar = 200 µm.

Conclusions

- High concentrations of valproic acid exhibited organoid neurotoxicity while high concentrations of gabapentin did not demonstrate neurotoxicity after 30 days of growth
- Cerebral organoids responded to known neurotoxic substances as clinically expected and can provide a biological model for screening human developmental neurotoxicity thereby improving prenatal care

Future Goals

- Refine methods in order to biologically analyze the drug and dose dependent reactions of specific neurodevelopmental stages of fetal brain development[2]
- Fully scale methods to efficiently screen additional pharmaceutics

References & Acknowledgments

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