A Human Pluripotent Stem Cell-Based Assay Accurately Predicts the Developmental Toxicity Potency for a Series of Valproate Analogues

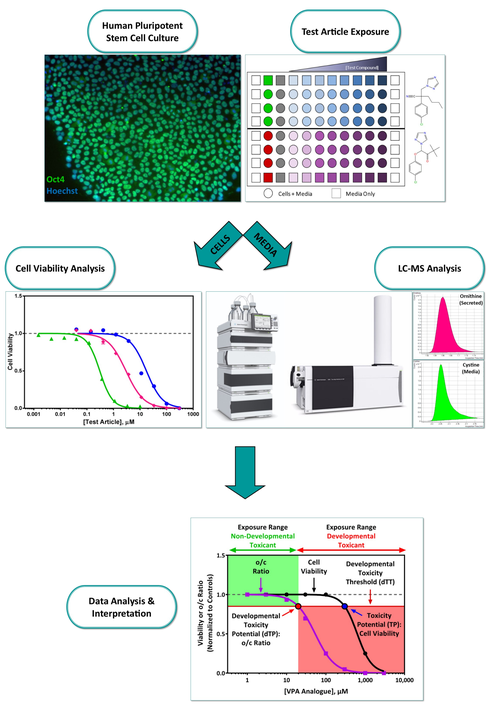
Jessica Palmer[1], Robert Burrier[1], Elizabeth Donley[1], Fred Kirchner[1], Dinant Kroese[2], Regina Stöber[3], Nicole Kleinstreuer[4]

[1]Stemina Biomarker Discovery, Madison, WI, United States; [2]The Netherlands Organization for Applied Scientific Research (TNO), The Hague, Netherlands; [3]IfADo – Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany; [4]National Toxicology Program Interagency Center for Evaluation of Alternative Toxicological Methods, Research Triangle Park, NC, United States

Abstract

- Regulatory acceptance of alternative methods for toxicity testing remains a challenge despite international efforts to reduce animal use.
- To address this, multiple agencies are working to develop a framework to implement the use of new approach methodologies for assessing the effects of chemical exposure on human health, such as the recently published ICCVAM "Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States" and EU-ToxRisk project.
- The EU-ToxRisk project has developed several case studies to address this issue. One of these case studies investigates the teratogenic potency of several valproate (VPA) analogues.
- The devTOX *quick*Predict platform (devTOX^{qP}) is an *in vitro* human pluripotent stem (hPS) cell-based assay that predicts the developmental toxicity potential of chemicals based on changes in hPS cell metabolism. Historical data has shown that the assay can accurately predict the developmental toxicity potential of diverse set of chemicals with known human and/or rodent *in vivo* developmental toxicity outcomes (N=111, 86% accuracy, 84% sensitivity, 87% specificity).
- The assay is being used by multiple industries and, of note, by the United States Environmental Protection Agency (EPA) and National Toxicology Program (NTP) in support of Tox21.
- In this study, we report the results from the devTOX^{qP} platform on eight VPA analogues included in the EU-ToxRisk case study. The hPS cell-based assay was used to rank their developmental toxicity potential *in vitro*. All of these analogues have published developmental toxicity potency data in an *in vivo* NMRI exencephaly-mouse model.

Methods



- Human induced pluripotent stem (iPS) cells (Cell Line: HYR0103, derived from primary hepatic fibroblasts; ATCC) were maintained in the undifferentiated state in mTeSR1 (StemCell Technologies) on Matrigel (Corning).
- Cells were plated in 96-well plates and exposed to 8 concentrations of each test article (1-3,000 µM) for 48 hours. Media ± test article were replaced approximately every 24 hours.
- Spent media from the last 24-hour treatment period was collected and filtered to remove molecules >3 KDa.
- Cell viability was assessed after sample collection using the CellTiter-Fluor Cell Viability Assay (Promega).
- Samples were analyzed with UPLC-ESI-TOF-MS to determine ornithine (ORN) and cystine (CYSS) levels.
- Non-linear dose-response curves analysis for the o/c ratio, ornithine and cystine response and cell viability were fit with GraphPad Prism.
- The developmental toxicity potential (dTP, o/c ratio) and toxicity potential (TP, cell viability) concentrations were predicted from the respective dose-response curves using the iPS cell developmental toxicity threshold (dTT, 0.85).

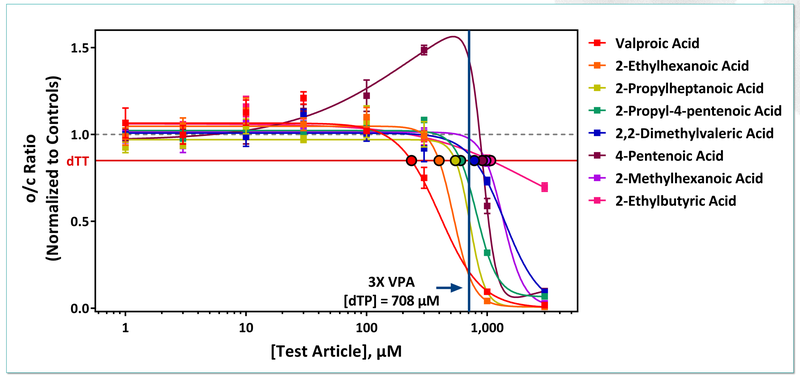
Results

devTOX^{qP} Results are Concordant with Available *In Vivo* Potency Data

Compound	CAS	Structure	Molecular Weight	devTOX^{qP} dTP (µM)	$\frac{dTP_{Analogue}}{dTP_{VPA}}$	*In Vivo* Potency[a]
Valproic acid (VPA)	99-66-1		144.21	**236**	**1.0**	+++[1,2,3]
2-Ethylhexanoic acid (2EHA)	149-57-5		144.21	**399**	**1.7**	+[4]
2-Propylheptanoic acid (2PHA)	31080-39-4		172.26	**546**	**2.3**	+++[1]
2-Propyl-4-pentenoic acid (4-ene-VPA)	1575-72-0		142.20	**604**	**2.6**	++[1,2,3]
2,2-Dimethylvaleric acid (2,2DVA)	1185-39-3		130.18	**784**	**3.3**	-[2,3]
4-Pentenoic acid (4PA)	591-80-0		100.12	**913**	**3.9**	-[2,3]
2-Methylhexanoic acid (2MHA)	4536-23-6		130.18	**976**	**4.1**	-
2-Ethylbutyric acid (2EBA)	88-09-5		116.16	**1,071**	**4.5**	-[2,3]

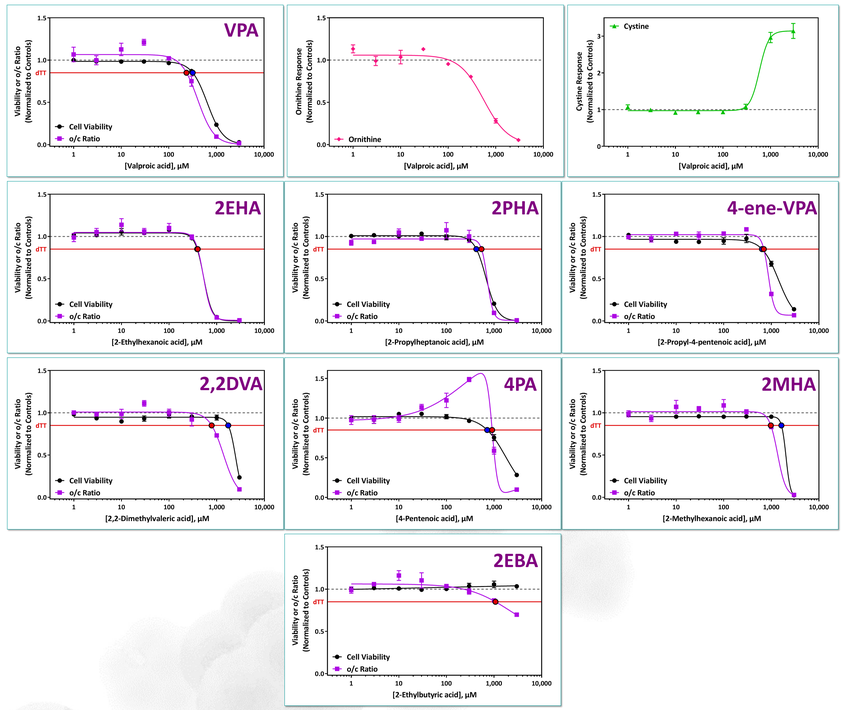
[a]Potency relative to VPA based on results in the NMRI exencephaly-mouse model using decision criteria in Eikel et al.[1]

VPA Analogues have Varying Potency in Human Induced Pluripotent Stem Cells



- Relative potency of VPA analogues can be categorized into two groups based on the ratio of the analogue dTP to VPA's dTP.
  - ◊ Analogues with high potency *in vivo* elicited a response in human iPS cells <3-fold of the VPA dTP concentration.
  - ◊ The dTP for analogues with little to no effect *in vivo* was >3-fold higher than VPA's dTP.

VPA Analogues Elicit a Dose-Dependent Response in Ornithine and Cystine



Conclusions

- The devTOX^{qP} *in vitro* hPSC assay was used to predict the developmental toxicity potential of a series of VPA analogues.
- The potency ranking from the devTOX^{qP} assay was consistent with observed developmental toxicity potency *in vivo*.
  - ◊ VPA was the most potent analogue in the devTOX^{qP} *in vitro* assay, which is concordant with its potency *in vivo* compared to the other analogues tested.
  - ◊ Analogues with *in vitro* activity at concentrations within 3-fold of VPA were also developmentally toxic *in vivo*, while those that had activity at higher *in vitro* concentrations had little to no effects in the mouse model.
- These results provide a human-relevant endpoint that could inform chemical prioritization and risk assessment.
- *Future Directions:*
  - ◊ *In vitro* to *in vivo* extrapolation to incorporate pharmacokinetics and predict relevant doses.
  - ◊ Compare results obtained here to other assay systems testing the VPA analogues as part of the EU-ToxRisk case study.
  - ◊ Joint publication with EU-ToxRisk following initial VPA analogue case study publication.

Funding

Research reported in this presentation was supported by NIEHS/NIH/HHS contract HHSN273201400027C.

References

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