

Neuronal calcium oscillations for preclinical seizure risk evaluation

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Abstract

Drug induced seizure or convulsion can be due to direct or indirect activity on central nervous system (metabolic impairment, blood brain barrier alteration...). **Epileptogenic and seizure risk prediction is a regulatory request and of major importance for any compound at preclinical stages.** The different available models mainly involve animals; although reliable, their use at early stage maybe limited since resource, time and compound consuming.

We are therefore developing a **new phenotypic functional in vitro model using neuro-glia cultures** that are able to develop a mature functional network. It allows us to follow its integrated synaptic activity by measuring the **kinetic of intracellular calcium events** that reflect potential for physiological seizure state (*DeLorenzo et al. 1998*). Measurements are performed using the FDSS μ Cell apparel (Hamamatsu©) with an intracellular calcium probe. **This model, able to detect known epileptogenic compounds** (Low magnesium, PTZ and 4AP) while also evaluating their mechanism of action (TTX, Mg, NMDA, etc.), will allow us to evaluate a potential risk.

Functional in vitro Model for seizure risk evaluation

Rodent Brain Primary cells Extraction from local Animal Research Welfare facilities

In vitro neuro-glia maturation and principle

Functional network | Synchronous NMDAR mechanism

Neuro-Glia Culture

Calcium Sensor 1h (Fluo-4, 8)

Wash and 0,1mM Mg medium

Drug injection

Calcium oscillation Neural Synchronicity

Calcium oscillations optimization

- brain structure used for Cell dissociation (cerebellum cortex hippocampus striatum)
- Cell density used
- Amount of glial cell
- In vitro maturation duration of synaptic network 12-27days
- Parameters measured
 - Number of oscillations
 - Amplitude of oscillations
- Comparison before and after injection
- Replicate and statistical test to be adapted (8 replicates on 3 plates and Anova test) and risk scoring

3 model of response

1/ Seizure like activity

2/ No activity

3/ Blocking like activity

Validation of the model & results: Magnesium, Dizocilpin, CNQX, 4Aminopyridin, pentylenetetrazol

Mg

MK-801

4-AP

PTZ EC50 : 3µM

4-AP EC50 : 30µM

Internal trial 12 compounds evaluation

ID	Structure	Response curve	Neural liability	Risk
B	CVL	reverse sigmoid	blocking like	Orange
	CTX	linear	none	
C	CVL	reverse sigmoid	blocking like	Red
	CTX	reverse sigmoid	blocking like	
F	CVL	reverse sigmoid	blocking like	Orange
	CTX	reverse sigmoid	none	
H	CVL	reverse sigmoid	blocking like	Orange
	CTX	linear	none	
8 CPD	CVL	linear	none	Green
	CTX	linear	none	

Outcomes

Oscillation ratio & EC50 = risk scoring:

- No effect
- >50% = increase (epileptogenic risk)
- <50% = decrease (blocking like risk)

Conclusion

This test, while promoting the 3R (Replacement Reduction Refinement of animal use), will allow a **quick evaluation of the seizure risk early** in drug development before progressing to other in vitro or in vivo assays.

Model Perspectives

The planned use of neuro-glia culture from **human iPSC (Induced Pluripotent Stem Cells)** and **3D Organoid Neurosphere** will lead to a better translation of drug risk evaluation to human safety.

- In vitro calcium oscillation
 - Dose level of risk
 - If positive, further tests available
- Ex vivo
 - Slice electrophysiology in rodent
- In vivo
 - Zebrafish activity test
 - PTZ induced convulsion in rodent
 - Rodent EEG telemetry

DeLorenzo RJ, Pal S, Sombati S. (1998) Prolonged activation of the N-methyl-D-aspartate receptor-Ca²⁺ transduction pathway causes spontaneous recurrent epileptiform discharges in hippocampal neurons in culture. Proc Natl Acad Sci U S A. 1998 Nov 24;95(24):14482-7
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