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Development of a Seizure Assessment Model Using Human iPSC-Derived Neural Cells.

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Background

- To design drug compounds without the risk of seizurogenicity is one of the significant issues for drug discovery. Although the risk is usually assessed by animal studies, there are practical difficulties such as the difficulty in assessing seizures through behavioral observations or occurrence of spontaneous seizure.
- In vitro assessment of seizurogenicity using rat primary neural cells or human induced pluripotent stem cell (hiPSC)-derived neural cells have been reported recently. However, verification of the assay is not enough because of the lack of knowledge about spices differences, lot-to-lot differences in cells or the effect of variable experimental settings.

Purpose of research

We established a seizures model using a co-culture system of hiPSC-derived neurons and astrocytes.
This study tested whether the model could detect effects of various compounds with different mechanisms under single or cumulative exposure and whether seizure risks could be identified at clinically toxic concentration ranges.

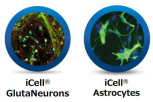
Materials and Methods

Measuring equipment

✓MEA：Maestro Pro (Axion BioSystems)
✓MEA plate: Cytoview (Axion BioSystems)

Reagents

✓Cell：iCell® GlutaNeurons, iCell® Astrocytes (FUJIFILM Cellular Dynamics, Inc.)
✓Coating agent：0.1% polyethylenimine
✓Media：BrainPhys Neural Medium with N2, iCell Neural Supplement B, iCell Nervous System Supplement, and PenisilIn-Spreptomycin



AssayProtocol

Data analysis

Test Compounds

Compounds	Mechanism of Action	Seizure lisk	Clinical side effects
Picrotoxin (PTX)	GABA antagonist	Positive	No data
Pentylenetetrazole (PTZ)			No data
Amoxapine (AXP)	D2R antagonist		No data
Diphenhydramine (DPH)			Toxic range 1.00~4.00 μg/mL [1)]
Chlorpromazine (CPZ)			Toxic range 750 ug/mL~ [2)]
Linopiridine (LPD)	K^+channel blocker		No data
4-Aminopyridine (4-AP)			No data
Pilocarpine (PILO)	M2R agonist		Lethal dose 60 mg [3)]
Theophylline (TPH)	A1R inhibitor		Toxic range 25 ug/mL~ [4)]
Strychnine (STRY)	GLR antagonist		No data
Acetaminophen (AAP)	Antipyretic agent	Negative	No data

1. Journal of Japanese Society for Emergency Medicine, 23, 611-615 (2020).
2. CONTOMIN SUGAR-COATED TABLETS Interview Form Version 14 (2024)
3. https://anzeninfo.mhlw.go.jp/anzen/gmsds/92-15-7.html
4. Theophylline SR Capsules 100mg [SANDOZ] Interview Form Version 2 (2024)

Result

Summary of Data

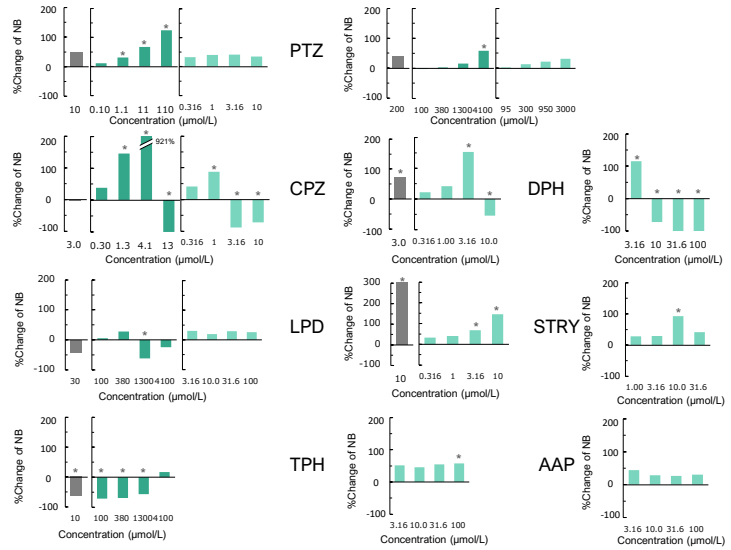
		Single Dose assay				Multiple Dose by Cumulative Addition							
		Study A				Study B				Study C			
		Conc. μmol/L	Spikes times/10min	Bursts times/10min	NB times/10min	Conc. μmol/L	Spikes times/10min	Bursts times/10min	NB times/10min	Conc. μmol/L	Spikes times/10min	Bursts times/10min	NB times/10min
PTX	GABA antagonist	0	31953	265	16	0	19872	167	9	0	73123	658	46
		10	35316	393	23	0.10	22832	187	10	0.316	83356	678	49
						1.1	22587	184	11	1	88738	711	52
						11	24375	226	13	3.16	86872	692	51
						110	25219	262	16	10	87289	695	51
PTZ		0	19675	229	15	0	15215	156	9	0	70848	603	40
		200	19616	275	20	100	14746	157	10	95	67046	581	38
						380	12461	152	10	300	74769	638	42
						1300	11435	167	11	950	79024	637	44
						4100	10927	181	14	3000	74230	685	50
AXP	D2R antagonist	0	19675	229	15	0	13624	150	10	0	66919	551	43
		3.0	29559	947	14	0.30	14765	205	14	0.316	83003	651	52
						1.3	17039	305	24	1	101995	858	69
						4.1	21139	604	100	3.16	133739	1709	5
						13	1192	0	0	10	66150	1943	11
DPH		not test				not test				0	44236	483	41
										3.16	38712	711	72
										10.0	36148	1165	11
										31.6	625	0	0
										100	0	0	0
CPZ		0	31953	265	16	not test				0	52768	533	39
		3.0	40035	960	27					0.316	48889	549	41
										1.00	54288	716	55
										3.16	20415	660	91
										10.0	11412	241	16
LPD	K+channel blocker	0	19675	229	15	not test				0	62879	474	40
		10	15466	411	60					0.316	73063	564	49
										1	53037	473	51
										3.16	46281	550	61
										10	46322	794	93
4-AP		0	31953	265	16	0	9842	110	8	0	40797	489	46
		30	17931	141	9	100	8507	104	10	3.16	45093	429	43
						380	8718	102	13	10.0	41876	446	46
						1300	9181	20	4	31.6	10308	277	46
						4100	5302	10	7	100	6914	203	43
PILO	M2R agonist	0	31953	265	16	0	12857	139	10	not test			
		10	19242	125	6	100	9666	37	3				
						380	9526	50	3				
						1300	7641	53	4				
						4100	4593	80	11				
TPH	A1R inhibitor	not test				not test				0	45615	589	58
										3.16	39114	479	51
										10.0	39395	521	56
										31.6	15165	319	55
										100	13768	297	54
STRY	GLR antagonist	not test				not test				0	49330	478	41
										1.00	54247	490	43
										3.16	51216	552	50
										10.0	18334	481	69
										31.6	12935	511	48
AAP	Antipyretic agent	not test				not test				0	46550	522	50
										3.16	48164	482	48
										10.0	46458	489	50
										31.6	16796	367	45
										100	15554	353	45

: Significant positive differences between compound and vehicle (DMSO) (Dunnett's test, p<0.05)
: Significant negative differences between compound and vehicle (DMSO) (Dunnett's test, p<0.05)

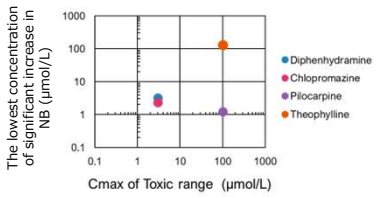
✓ The risk of seizurogenicity was correctly assessed for 8 compounds.
✓ The NB was the most sensitive parameter in multiple addition assay.
✓ Compounds without clinical data reacted at concentration ranges similar to those reported previously.
✓ AAPs that have not been reported to cause seizures clinically were negative.
✓ When NB occurs at a high frequency, it is not correctly detected.
✓ GABA antagonists (PTX, PTZ) and 4-AP yielded inconsistent results.

%Change of NetworkBurst

* : Significant differences between compound and vehicle (DMSO) (Dunnett's test, p<0.05)

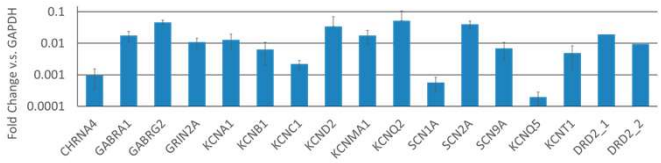


Correlation of Clinical information



✓ The Compounds with high brain penetration (DPH, CPZ, and TPH) increase in NB was observed at concentration ranges reported to toxicity range.

q-PCR



✓ mRNA was extracted from the cells four weeks after seeding, and q-PCR was performed.
✓ The expression of genes associated with seizurogenicity was confirmed.

Conclusion

In our developed a seizure model, we detected the effects of test compounds with different mechanisms under single or cumulative exposure. We also confirmed that seizure risks could be identified at concentration ranges reported to cause clinical toxicity. Moving forward, we aim to establish reproducibility.