

Aggregating brain cell cultures as a model for acute neurotoxicity testing



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WP 7.1 Partner no. 20



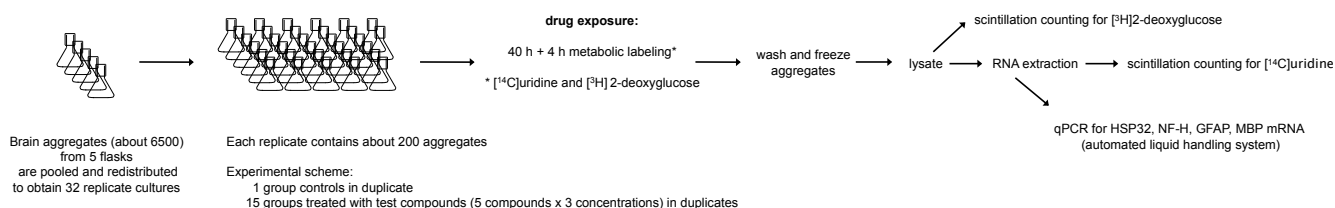
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OBJECTIVES

Simple cell line-based test systems often fail to detect organ-specific toxicity. The goal of the present work was to find complementary in vitro systems able to detect nervous system-specific toxicants. Aggregating brain cell cultures were examined for their suitability as a model to alert for toxicants affecting specifically the nervous system. A set of 57 reference compounds was used for the evaluation.

METHODS

Aggregating brain cell cultures prepared from 16-day fetal rat brain were used as the test system, and a high-content approach was chosen for the detection of adverse effects. Replicate cultures were exposed to the test compounds for 44 h, and the adverse effects were analyzed on 6 endpoints, e.i., the expression levels of 4 genes (GFAP, MBP, NF-H, HSP32) determined by qRT-PCR, the rate of [¹⁴C]uridine incorporation into RNA, and the rate of [³H]2-deoxyglucose uptake reflecting activity-dependent glucose consumption.



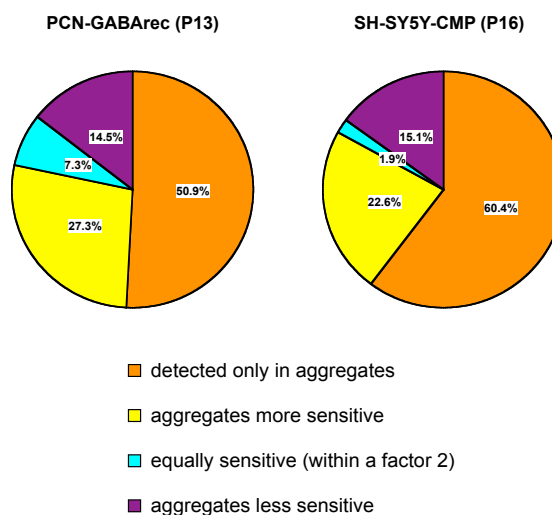
RESULTS

The 59 reference compounds have been tested at least three times. All data have been reported to Acubase. Compound concentrations causing significant adverse effects in aggregate cultures were compared with those found in other systems. Figure 1 shows compounds regarded as outliers, for which >10-fold higher sensitivity was found in aggregate cultures as compared to two cell line-based routine test systems. Based on this criterion, 46% outliers were found compared to the 3T3-NRU method, and 37% outliers compared to the NHK-NRU method. Compared to the CYTOMIX system (P9), 20% outliers were found (not shown). A comparison was also made with the two most complete datasets of WP 7.1 (Figure 2).

Figure 1: Aggregates compared to two routine test systems

Concentration range of adverse effects	3T3-NRU outliers	NHK-NRU outliers
< 10 ⁻⁶ M	Ochratoxin A Cycloheximide Digoxin Glufosinate Pentachlorophenol Arsenic trioxide	Ochratoxin A
	Mercury chloride	Glufosinate Pentachlorophenol Arsenic trioxide Cadmium chloride Mercury chloride
10 ⁻⁶ – 10 ⁻⁴ M	Cyclosporin A Diquat dibromide Epinephrine	Epinephrine 17 α -Ethinylestradiol
	Amiodarone 5-Fluorouracil Physostigmine Lindane Diazepam Parathion Sodium selenate Acrylaldehyde Dichlorvos Carbamazepine Pyrene Malathion	5-Fluorouracil Physostigmine Lindane Diazepam Parathion Dichlorvos Carbamazepine
2x10 ⁻⁴ – 6x10 ⁻² M	Phenobarbital Sodium valproate	Phenobarbital Sodium valproate Chloral hydrate 2,4-Dichlorophenoxyacetic acid Diethylene glycol
	Diethylene glycol Methanol	Diethylene glycol Lithium sulfate
	46%	37%

Figure 2: Aggregates compared to two other systems of WP 7.1



CONCLUSIONS

1. Aggregating brain cell cultures offer a robust, reproducible, and realistic model to assess the CNS-specific toxicity of chemicals.
2. The concentration-dependent adverse effects were generally in good agreement with the known toxicity in vivo.
3. Outliers in aggregate cultures appear to be compounds acting at the level of the whole organism (e.g., ethanol, atropine, and nicotine).
4. The sensitive detection of adverse effects requires a set of endpoints representing specific structural and functional traits.
5. The actual throughput can be increased by further automation and upscaling.