

Assessment of neurochemical end-points in primary neuronal cultures to predict acute toxicity of neurotoxic compounds

Cristina Suñol, Daniel A. García, Susana Iraola, Zoila Babot

Department of Neurochemistry. Institut d'Investigacions Biomèdiques de Barcelona.

Consejo Superior de Investigaciones Científicas, CSIC. Rosselló 161. Barcelona.

csenqi@iibb.csic.es

Acute systemic toxicity correlating to adverse neuronal function is mainly a result of over-excitation or depression of the CNS or PNS, generating symptoms such as lethal convulsions, respiratory arrest or unconsciousness *in vivo*. The major molecular mechanisms involved in stimulatory and inhibitory/depressive activity of the CNS and PNS, as well as brain lesions, include GABAergic, glutamatergic and cholinergic neurotransmission. Crucial molecular end-points for acute neurotoxicity are: voltage-operated ion channels and ligand operated receptors, including ionotropic GABAA, NMDA and AMPA/kainate, and nicotinic acetylcholine receptors; transport of aminoacidergic and aminergic neurotransmitters; enzymes involved in neurotransmitter synthesis and degradation. Other mechanisms with less neuronal specificity, but with absolute requirement for maintained CNS functionality are: cell energy status; cell membrane and mitochondrial membrane potentials; intracellular calcium homeostasis, [Ca²⁺]_i; and controlled production and inactivation of reactive oxygen species (ROS). It is expected that neurotoxic chemicals will produce their effect by different mechanisms. The evaluation of these mechanisms will allow establishing a hierarchy in the role of the different assays in their capacity of predicting the potential neurotoxicity of the compounds.

A set of compounds, covering pharmaceutical, industrial, biocide and drug abuse chemicals, have been tested against GABA_A receptor activity, GABA and glutamate transport and acetylcholinesterase activity in primary neuronal cultures. The results from these studies on neural mechanisms have been correlated to cell viability endpoints, which are independent of cell type or organ function. Thus, cell membrane disruption and cell viability have been used in the experimental models as non-neurotoxicity specific markers for comparison to the endpoints representing neurotoxicity.

The inhibition of GABA_A receptor-mediated chloride influx by atropine, carbamazepine, lindane, malathion, methadone and pentachlorophenol might result in seizures as they have been observed after severe human poisoning. The relative potency of 2-propanol and ethanol against potentiation of the GABA_A receptor correlates with the greater CNS depression produced by 2-propanol. Methadone and amphetamine inhibit [³H]GABA uptake but not [³H]aspartate uptake, methadone being more potent than amphetamine. Digoxin preferentially inhibited [³H]aspartate uptake. Only mercuric chloride, pentachlorophenol and sodiul lauryl sulfate (SLS) decreased cell viability, however pentachlorophenol and SLS were more potent at neuronal endpoints. The organophosphorus compounds dichlorvos and paraoxon, but not parathion inhibited acetylcholinesterase activity, suggesting that primary cortical neurons do not have metabolic capacity. Acetaminophen and acetylsalicylic acid did not modify any of the endpoints assayed. These drugs do not produce acute neurotoxic effects in humans. These results show that the *in vitro* evaluation of neural endpoints may identify compounds that produce acute neurotoxicity in humans, provided that relevant *in vitro* models for acute neural disfunctions are used.

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Poster

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