

THE USE OF THE HUMAN NEUROBLASTOMA SH-SY5Y CELL LINE FOR ESTIMATION OF ACUTE SYSTEMIC TOXICITY

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Acute systemic toxicity, expressed as human lethal blood peak concentration or the dose inducing 50 % lethality in an animal population (LD_{50}), can be estimated by general cytotoxicity tests using proliferating mammalian cell lines for 70-80 % of all chemicals. The cytotoxicity for the remaining chemicals over- or underestimate the LD_{50} values/human lethal blood peak concentrations because of their very specific molecular targets or toxicokinetic features in vivo. The objective of the EU funded integrated project "ACuteTox" is to develop a strategy in which organ-specific endpoints and toxicokinetic features are taken into consideration in the in vitro prediction of acute systemic toxicity. The human neuroblastoma SH-SY5Y cell line was used as a model for studies on neurospecific targets, which are known to be crucial for survival. All endpoints were investigated after short exposure times (minutes to an hour) at concentrations of the test chemicals that did not affect the cell viability, measured as cell membrane leakage of lactate dehydrogenase. The effects of 23-26 compounds (drugs, pesticides and industrial chemicals) were studied on the cell membrane potential (CMP), voltage dependent Ca^{2+} channels (VDCC), muscarinic acetylcholine receptor (mAChR) function, acetylcholinesterase (AChE) activity and noradrenalin uptake. The results showed that the CMP was altered by atropine, amphetamine, mercury chloride, methadone, nicotine, pentachlorophenol, sodium lauryl sulphate (SLS) and verapamil, whereas an effect on VDCC could be detected for amphetamine, atropine, colchicine, pentachlorophenol, SLS and verapamil. The mAChR function was measured as carbachol-induced increase in the intracellular free Ca^{2+} concentration. Amphetamine, pentachlorophenol, SLS and verapamil attenuated the carbachol response by 50% at concentrations about 1 mM, but the specific mAChR antagonist atropine had the same effect at 3 nM. Nicotine, caffeine, pentachlorophenol, methadone, mercury chloride, SLS and the specific inhibitors physostigmine, dichlorvos and malathion attenuated the AChE activity at significantly non-cytotoxic concentrations in SH-SY5Y cells after 60 minutes of exposure. Parathion did not inhibit the AChE activity after 60 minutes exposure, but after 48 hr, indicating that oxidation of parathion to the active inhibitor paraoxon took place in the cell culture. This phenomenon was also observed for malathion, which displayed a lower EC_{50} value after the prolonged exposure time. The noradrenalin uptake was affected by atropine, caffeine, carbamazepine, amphetamine, diazepam, isopropanol, methadone, SLS and verapamil. A comparison of the active concentrations with the basal cytotoxicity measured by the validated neutral red uptake assay in mouse fibroblast 3T3 (3T3-NRU) cells after 72 hrs of exposure, indicated that the AChE assay is useful for detection of AChE inhibitors and possibly also AChR ligands. The CMP was the most sensitive endpoint for nicotine, indicating activation of nicotinic AChR at 100 times lower concentration than the concentration inducing basal cytotoxicity in 3T3 cells. The VDCC endpoint was useful as an alert only for verapamil and the mAChR function was only specifically affected by atropine. The noradrenalin uptake indicated a clear alert for amphetamine and methadone, which was expected, but not for the other test compounds. These results indicate that the usefulness of these endpoints in a general test battery for estimation of acute systemic toxicity is limited, except for AChE activity measurements. However, the results clearly showed that the compounds with known mechanisms (e.g. atropine, verapamil, amphetamine and methadone) displayed expected effects on their specific endpoints.