Umeå University Medical Dissertations, New Series No 1877

In vitro cellular models for neurotoxicity studies:
neurons derived from P19 cells

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt försvar i Hörsal D, Unod T9, byggnad 1D, NUS,
fredagen den 28 april, kl. 9:00.
Avhandlingen kommer att försvaras på engelska.

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Department of Pharmacology and Clinical Neuroscience
Umeå University
Umeå 2017

Organization
Umeå University
Department of Pharmacology
and Clinical Neuroscience

Document type
Doctoral thesis

Date of publication
6 April 2017

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Title
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Abstract
Humans are exposed to a variety of chemicals including environmental pollutants, cosmetics, food preservatives and drugs. Some of these substances might be harmful to the human body. Traditional toxicological and behavioural investigations performed in animal models are not suitable for the screening of a large number of compounds for potential toxic effects. There is a need for simple and robust *in vitro* cellular models that allow high-throughput toxicity testing of chemicals, as well as investigation of specific mechanisms of cytotoxicity. The overall aim of the thesis has been to evaluate neuronally differentiated mouse embryonal carcinoma P19 cells (P19 neurons) as a model for such testing. The model has been compared to other cellular models used for neurotoxicity assessment: retinoic acid-differentiated human neuroblastoma SH-SY5Y cells and nerve growth factor-treated rat pheochromocytoma PC12 cells. The chemicals assessed in the studies included the neurotoxicants methylmercury, okadaic acid and acrylamide, the drug of abuse MDMA ("ecstasy") and a group of piperazine derivatives known as "party pills". Effects of the chemicals on cell survival, neurite outgrowth and mitochondrial function have been assessed.

In Paper I, we describe a fluorescence-based microplate method to detect chemical-induced effects on neurite outgrowth in P19 neurons immunostained against the neuron-specific cytoskeletal protein βIII-tubulin. In Paper II, we show that P19 neurons are more sensitive than differentiated SH-SY5Y and PC12 cells for detection of cytotoxic effects of methylmercury, okadaic acid and acrylamide. Additionally, in P19 neurons and differentiated SH-SY5Y cells, we could demonstrate that toxicity of methylmercury was attenuated by the antioxidant glutathione. In Paper III, we show a time- and temperature-dependent toxicity produced by MDMA in P19 neurons. The mechanisms of MDMA toxicity did not involve inhibition of the serotonin re-uptake transporter or monoamine oxidase, stimulation of 5-HT_{2A} receptors, oxidative stress or loss of mitochondrial membrane potential. In Paper IV, the piperazine derivatives are evaluated for cytotoxicity in P19 neurons and differentiated SH-SY5Y cells. The most toxic compound in both cell models was TFMPP. In P19 neurons, the mechanism of action of TFMPP included loss of mitochondrial membrane potential. In conclusion, P19 neurons are a robust cellular model that may be useful in conjunction with other models for the assessment of chemical-induced neurotoxicity.

Keywords
Neurotoxicity, neurite outgrowth, P19 cells, SH-SY5Y cells, PC12 cells, methylmercury, okadaic acid, acrylamide, MDMA, piperazine-derived designer drugs.

Language
English

ISBN
978-91-7601-659-6

ISSN
0346-6612

Number of pages
67 + 4 papers