Lecture: alternative testing methods for toxicity to reproduction

Development of an *in vitro* test system for developmental neurotoxicity

Bastian Zimmer, Suzanne Kadereit, Philipp B. Kügler, Marcel Leist
University Konstanz (Konstanz) (DE)
e-mail: bastian.zimmer@uni-konstanz.de

Subject/Motivation/Background: Little is known about the effects on the human CNS development of most of the chemical substances we handle every day. Evidence is emerging that neurodegenerative diseases such as Alzheimer's disease might be correlated with exposure to toxicants in early life stages and early exposure e.g. to lead has affected the development of millions of children (Landrigan et al., 2005). Therefore we aim to establish a neurodevelopmental toxicity assay based on embryonic stem cells (ESC).

Material and Methods: To differentiate mESCs into neurons a protocol published by Ying and Smith in Methods of Enzymology in 2003 was slightly modified. Successful differentiation was checked by RT-PCR and Immunofluorescence analysis. 5-Fluorouracil, CH_3HgCl and PenicillinG were used as model substances to test the potential of our system to predict neurodevelopmental toxicity. Sublethal concentrations of these substances were applied to the culture for the last 6 days of differentiation. Effects were analyzed by RT-PCR and Western Blot.

Results: Differentiation of Tubulin, III−βmESC into neurons resulted in a percentage of 70% neurons (N-CAM), 15% nestin-positive precursor cells, and approximately 10% glial cells. The latter were located within rare and small islands within an even layer of tubulin positive cells to mature neurons was shown III−βneurons. Maturation of by the presence of several markers such as, NeuN (postmitotic), SNAP25 (pre-synaptic), PSD95 (postsynaptic), SV2 (synaptic vesicles-2), NMDAR1 (postsynaptic), tyrosin hydroxylase (dopaminergic neurons), glutamic acid decarboxylase (GABAergic neurons). The staining pattern of the analyzed markers within ESC-derived neurons was similar to that of primary neurons (CGCs) isolated from mice. The whole differentiation process was monitored by RT-PCR and Western blot to determine the time window of the onset of neuronal differentiation. Toxic substances were added at that point in time to the culture and marker expression was analyzed. RT-PCR analysis showed a significant difference in the synaptophysin, N-CAM, nestin and MAP2 mRNA content of CH_3HgCl asβtreated cells compared to untreated cells. Glial markers like GFAP and S100 well as the endodermal marker GATA-4 and the mesodermal marker brachyury were not affected by the treatment with CH_3HgCl. Treatment with the known strong teratogen 5-FU significantly reduced the amount of the endodermal marker GATA-4. Viability of the cells was not affected by the treatment as confirmed by MTT assay and morphological observation.

Discussion: We show here the differentiation of mESCs into neurons. We additionally showed that our system can detect the impact of toxicants at sublethal concentrations. This effect was restricted to the neuronal lineage, as glia and markers for the other two germ layers were not affected. We therefore believe that our system can detect toxic effects on the development and functionality of neurons.

Keywords: mESC, development, neurotoxicity, methylmercury