Poster: alternative testing methods for toxicity to reproduction

The human dopaminergic neuronal cell line LUHMES as *in vitro* model for Parkinson´s disease

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Background: Parkinson´s disease is characterized by a gradual degeneration of dopaminergic neurons in the substantia nigra. Dopaminergic neurons are continuously exposed to elevated oxidative stress conditions due to the unstable neurotransmitter dopamine that can easily undergo oxidation to form superoxide and a quinine-form capable to react with cysteine residues in proteins or with glutathione to form dopamine-conjugates. For investigations on the molecular events occurring under these conditions, as well as for the validation of potential pharmacological interventions, an experimental human *in vitro* model that closely resembles the characteristics of dopaminergic neurons *in vivo* is desired. This *in vitro* model would not only allow to significantly reduce the use of *in vivo* animal testing, but concomitantly would have the advantages to allow studies on molecular events occurring in neurodegenerative disorders in a human system.

Materials and Methods: LUHMES are human mesencephalic cells conditionally immortalized with a v-myc retroviral vector. In this system, tetracycline shuts down v-myc expression and allows differentiation into dopaminergic neurons. Tetracycline in combination with dbcAMP and GDNF (glial cell-derived neurotrophic factor) leads to a differentiation into dopaminergic cells within 3-4 days, as shown by the expression of specific markers such as tyrosine hydroxylase or dopamine transporter (DAT).

Results: LUHMES cells were validated with respect to their response toward the parkinsonian toxins MPP^+ and methamphetamine (METH)/$Fe2^+$. In both cases, a time-dependent degradation of neurites, accompanied by a loss of cellular ATP and GSH, and increased formation of radical species was observed. These effects were only detected in fully differentiated cells, whereas undifferentiated LUHMES demonstrated no significant response to the same toxic insult. The neurodegenerative effects observed were partially prevented or delayed by co-incubation with the mixed lineage kinase inhibitor CEP1347, or by inhibition of poly-ADP-ribose polymerase (PARP). The involvement of dopamine in the neurodegenerative process was further underlined by application of dopamine transporter, or tyrosine hydroxylase inhibitors that significantly protected against MPP^+-induced degeneration.

Discussion: Parkinson´s disease and other neurodegerative disorders such as Alzheimer´s disease or multiple sclerosis are one of the most challenging health issues in the aging populations of developed countries. It can therefore be assumed that efforts of the pharmaceutical industry within this field will increase dramatically within the next decades. These research projects will create a massive rise in the demand for reliable and representative test systems. The most widely used model for this purpose however is still the laboratory animal. The herein introduced human neuronal cell line closely reflects the unique properties of dopaminergic cells *in vivo*. This model can not only serve for basic research on the events occurring in neurodegenerative diseases, but can also be used as a screening system within neurotoxicological testing programs.

References

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