Rotenone-induced toxicity in human iPSC derived dopaminergic neurons (iCell®DopaNeurons): a cellular model to discover novel neuroprotective drugs

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**Aim of the study:** set up a compound-rescue assay in a physiologically relevant cell system to screen for novel neuroprotective drugs

iCell®DopaNeurons characterization provided by FCDI

iCell®DopaNeurons are a fully differentiated, highly purified human population of midbrain dopaminergic neuron derived using FCDI’s proprietary differentiation and purification protocols.

Comparison of culture in BrainPhys™ vs FCDI’s maturation medium

BrainPhys™ Neuronal Medium is a serum-free, low-glucose medium described for culture and maturation of hPSC-derived neurons. FCDI’s maturation medium (FCDI-MM) has a 10X concentration of glucose compared to BrainPhys™, which could potentially make cells less sensitive to a toxic insult.

**Rotenone-induced mitochondrial toxicity**

Mitochondrial dysfunction and oxidative stress are pathophysiological mechanisms implicated in many neurodegenerative experimental models. Different molecules impairing the mitochondrial membrane potential (MMP) were tested to check their toxic effect on iCell®DopaNeurons viability: Antimycin A, that inhibits mitochondrial respiration complex III; Oligomycin as a complex V inhibitor and Rotenone, a complex I inhibitor.

**Neuroprotection by Carnosic acid (CA)**

Carnosic acid is a natural compound found in rosemary and sage that easily crosses the blood-brain barrier. CA is pro-electrodephile agent has been shown to be a neuroprotective agent against oxidant/stressful conditions both in vitro and in vivo experimental models.

**Carnosic acid**

Carnosic acid (CA) rescue effect RT-Glo

One hour before injury, CA (30 μM) was added and fixed on EC2, of Rotenone was subsequently applied, viability was measured at 48h using RT-Glo. MeanSEM is shown (n=4). Replicate is a independent experiment.

**Conclusions**

iCell®DopaNeurons represent a suitable model to further investigate the neurotoxicity mechanism of Rotenone injury and to screen compounds with neuroprotective potential for neurodegenerative diseases, using CA as a reference neuroprotective compound. For drug discovery purposes a high-throughput phenotypic assay using TMRE can be multiplexed with a viability readout RT-Glo.

**Perspectives**

- complete the assay set-up (Z factor)
- screening of LOPAC®1280
- further characterization of positive hits
- assay set-up using cells with disease-linked mutations

**Materials and methods**

Cells were thawed following manufacturer’s instructions for use and recommended precultures. About 15,000 viable cells/well were plated in 24 Greiner FCDI plates, previously coated once with polyornithine and overnight with laminin (both from Sigma-Aldrich). During culture, half of the medium was replaced twice a week.

**References**