A VERSATILE MICROGLIA-NEURON CO-CULTURE SYSTEM FOR THE IDENTIFICATION OF ANTI-INFLAMMATORY NEUROPROTECTANTS - APPLICATION TO SCREENING OF NATURAL COMPOUNDS

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Introduction: In Alzheimer’s disease, activated microglia secrete cytokines, including IL-1, IL-6 and TNF as well as reactive oxygen and nitrogen species (ROS/RNS). These factors contribute to alterations in neuronal glucose uptake, inhibition of mitochondrial enzymes, impaired axonal transport, and synaptic signaling. In addition, ROS act as signaling molecules in pro-inflammatory redox-active signal transduction.

Aims: To establish a high throughput screening system for the discovery of novel anti-inflammatory and neuroprotective compounds, we have constructed “Enhanced Green Fluorescent protein” (EGFP) expressing murine Neura2A cells and set up a microglia/neuron co-culture system with these EGFP expressing neuronal cells.

Methods: As markers of inflammation, NO and TNF were measured. As a marker of cell viability, loss of GFP mediated fluorescence was determined. For the measurement of the size of the dendritic tree, fluorescence microscopy and high content image analysis (HCA-Vision, CSIRO) were employed.

Results: We show that microglial activation leads to neurite retraction and neuronal cell death, which can be conveniently visualized in a 96 well plate format. Moreover, we have used this system to test complex herbal extracts, polyphenolic compounds and thiol based antioxidants including lipoic acid for their ability to downregulate inflammatory markers and to protect neurons against microglial insult. The most potent compounds in these assays were the polyphenols apigenin, diosmetin and carnosic acid with EC50 values in the range of 5-10 µM.

Conclusions: This system is useful to screen a variety of known and novel compounds and extracts (alone and in combination) for the treatment of the inflammatory aspect of AD.