Brain Diseases

ISCHEMIA

Background

Brain ischemia is a condition where there is insufficient blood flow to the brain to meet metabolic demand. This leads to poor oxygen supply or cerebral hypoxia, resulting in the death of brain tissue or cerebral infarction / ischemic stroke.

Pathology Model

Primary cortical neurons isolated form Sprague Dawley rats will be exposed to oxygen glucose deprivation (OGD) for 1h in presence absence of CLIENT's compound and to reoxygenation for 24 hours as a model of cerebral ischemia. Complicating the biological scenario, as cerebral microenvironment plays a crucial role in pathogenesis of cerebral ischemia, neurons will be put in microfluidic communication with glia from different brain areas (such as cortex or hippocampus) and then subjected to OGD and reoxygenation following the protocol described above, in presence absence of CLIENT's compound.

.

Readouts

After reoxygenation the following parameters will be evaluated:

Step 1 - Direct Neuronal Damage

1. Morphological Characterization
    - Qualitative evaluation of neuronal cytoskeletal disruption
    - Quantitative evaluation of dendritic branching
    - Quantitative evaluation of neurite elongation modulation
2. Biochemical Characterization
    - Quantitative evaluation of neuronal cell death (i.e. PI/DAPI/Calcein AM)
    - Quantitative evaluation of caspase activation (i.e. 3 or 8 or 9)
    - Quantitative evaluation of DNA degradation (i.e. TUNEL staining)
3. Analysis of Oxidative Stress
    - Quantitative evaluation of total ROS production
    - Quantitative evaluation of NO production
    - Mitochondrial membrane potential (MMP) (JC-10 dye by flow cytometry)

ISCHEMIA

Step 2 - Glial Pro-Inflammatory Phenotype

1. Biochemical characterization
   - Quantitative evaluation of metabolic activity
   - Inflammatory cytokine production: (i.e. IL1 beta, TNFalpha, IL6)
   - Total ROS production
   - Quantification of NO production

2. Functional characterization
   - Quantitative evaluation of phagocytic potential
   - Quantitative evaluation of membrane permeability
   - Quantitative evaluation of microvesicle shedding