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Project title: Crosstalk between neuroinflammation and disruption of metabolism and redox homeostasis in models of Parkinson disease

Abstract

Maintenance of redox homeostasis is crucial to cell survival and is constantly controlled by a number of signaling pathways that are activated in response to stress and metabolism. Modifications of the redox homeostasis system is linked to a variety of pathological conditions, such as aging and age-related diseases. The overall goal of this project is to study the crosstalk between neuroinflammation and disruption of metabolims and redox homeostasis in models of Parkinson's disease (PD). To this end, we propose to use our PD model based on 6-OHDA injection in the basal ganglia to study the effect of the PD-related neurotoxin and neuroinflammation on neuroglial plasticity, metabolism and the redox balance. Specific cellular contributions (neuronal/glial) will be dissected using in-vitro PD models based on 6OHDA (primary neurons and astrocytes). These studies should provide an exhaustive picture of the biochemical events linking PD onset, neuroinflammation, and disruption of metabolism and redox homeostasis.

Background, aims and significance of the proposed work

Parkinson's disease (PD) is a chronic neurodegenerative disorders characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). Genetic and environmental factors linked to the onset of PD converge on oxidative stress and mitochondrial dysfunction (Ryan et al., 2015).

Moreover, increasing evidence supports the relevance of inflammatory processes in PD. Activated glial cells and upregulation of pro-inflammatory cytokines have been reported both in models and in PD patients (Gerhard et al., 2006; Hirsch et al. 2012). Neuroinflammation is sustained by microglia and astrocytic activation, leading to alteration of glial phenotype and function, disruption of synaptic plasticity and alteration of neurotrophic support (Colangelo et al., 2014). Although these changes represent a physiological response of CNS to minimize neuronal damage, sustained neuroinflammation provides detrimental signals that compromise astrocytic and neuronal functions and exacerbate neurodegeneration.

In addition to their role in inflammatory processes, astrocytes play a key role in synaptic function and neuronal metabolism through what is known as "neuro-metabolic coupling", connecting synaptic function with energy requirements (Magistretti PJ, 2006). Changes in the metabolism and redox homeostasis system have been linked to aging and to a variety of age-related pathological conditions. In particular, maintenance of redox homeostasis is crucial to neuronal survival and is constantly controlled by a number of signaling pathways that are activated in response to stress and metabolism.

We have recently constructed a comprehensive dynamic model of ROS management (Kolodkin/Westerhoff) showing the complex mitochondria-ROS network and its regulation by signaling pathways linked to antioxidant responses (Nrf2, DJ.1, NF-kB, …), redox homeostasis and cell viability. This model might be extended as to include its connection to the metabolic –redox networks.

The proposed studies will be essential to gain insights into mechanisms connecting oxidative stress and astrogliosis in PD models and their correlation with mechanisms determining mitochondrial and metabolic dysfunction.

Experimental plan

To investigate mechanisms linking neuroinflammation to mitochondrial dysfunction and disruption of redox metabolism in PD, we propose to use both the mice model of neurotoxin-PD and in-vitro studies on primary cultures of neurons and astrocytes for detailed characterization of processes.

PD mice receive one single injection of 6-OHDA and are daily monitored for motor functions and sacrificed at specific time-points (3, 7, 14, 21 days). Brain sections or specific tissues (CTX, SNc, Striatum) are then used for immunohistochemical (IHC) and biochemical analyses (**aims 1 and 2**). Primary cultures of neurons and astrocytes (prepared as described in Martorana et al., 2019) will be also used to obtain a detailed characterization of the specific cellular contributions (neuronal/glial) (**aim 3**).

On both models, we aim to study the effect of the PD-related neurotoxin and neuroinflammation on oxidative stress and alteration of neuroglial plasticity and mitochondrial dysfunction and metabolism. All together, these studies should provide an exhaustive picture of the biochemical and molecular events linking neuroinflammation with alterations of mitochondrial morphology and metabolism.

Specific objectives will include:

Aim 1. Evaluate mechanisms of synaptic disruption in the 6-OHDA-PD model

We have recently established a mice model of PD based on a single unilateral injection of 6-OHDA in the basal ganglia. Histological analysis of paraffin-embedded brain slices indicated the onset of microglial and astrocytic activation in the ipsilateral, but not in the contralateral side of the injection at early time-points. Moreover, motor tests showed that 6-OHDA mice present the typical PD deficit of motor function starting at 21 days post injection. We will need to run a second set of groups of mice with the same 6OHDA injection, and sham-operated mice as control, for the extended time-course (3, 7, 14, 21 and 35 days) for additional biochemical studies. After sacrificed, brains will be processed differently from the previous sets, i.e. they will be frozen. Brain sections of 25 µm thick cut on a microtome will be used for histochemical analysis that could not be performed on paraffin-embedded tissues, including some mitochondrial proteins (P-Drp1) and other phosphorylated proteins of the CaMK signaling, as well as synaptic components (glutamate/GABA, TH, DAT) and stress responses (P-JNK, Nrf2, DJ1).

Aim 2. Assess mechanisms of brain redox homeostasis and metabolism in the 6-OHDA-PD model

We propose to focus on the crucial role played by the redox couples NAD+/NADH and NADP+/NADPH, which regulate the cellular redox homeostasis, mitochondrial function and metabolism. The NAD+/NADH regulates the energy metabolism (glycolysis and the TCA cycle/oxidative phosphorylation), while the NADP+/NADPH is essential in maintaining the redox homeostasis and the biosynthesis of fatty acids and nucleic acids. The balance NADP+/NADPH is required to maintain Glutathione (GSH) in its reduced form. GSH is the most abundant antioxidant and is essential to maintain neuronal redox homeostasis. Alterations in NADP+/NADPH, GSH/GSSG and cysteine/cystine balance and metabolism can affect cell survival in response to a variety of stimuli or neurotoxins that affect cell metabolism and/or induce oxidative stress. This is particularly relevant for neuronal viability and brain function, due to its prevalent oxidative metabolism and accumulation of ROS. Therefore, the NADP+/NADPH, GSH/GSSG and cysteine/cystine balance/utilization may represents potential therapeutic targets.

To this end, adjacent brain slices (from the groups of mice in Aim1) will be used to assess components of the redox homeostasis, such as the NADP+/NADPH, GSH/GSSG and to study their connection with metabolic pathways / rewiring, and the cysteine/cystine balance.

Metabolic profiling will be performed on a separate set of mice that will go through the same 6OHDA injection (sham-operated mice as control) for the extended time-course for 3, 7, 14, and 21 days). After

sacrificed, brains will be used to dissect discrete brain areas (substantia nigra, striatum, cortex, and cerebellum as negative control), and immediately frozen. Samples will be then processed for metabolic profiling (in collaboration with SysBio-Metabolomics). Brain samples will be processes by standard procedures for metabolite extraction and metabolomic analysis (Metabolomics-SYSBIO platform) by liquid/gas chromatography coupled to a MS systems (6550 I-Funnel LC-QTOF and 7200 GC-QTOF-Agilent Technologies), followed by quantitation and pathways identification by Mass Hunter software - Agilent Technologies.

Aim 3. Detailed characterization of brain redox homeostasis and metabolism using in-vitro 6-OHDA-PD models

The specific cellular contribution (neuronal/astroglial) to disruption of redox homeostasis and metabolism will be evaluated by performing metabolic profiling on primary cultures of astrocytes treated with 6OHDA for 3-6-24h, and on primary neurons exposed for 6 or 24h to the conditioned medium from 6OHDA-treated astrocytes. The effect of the PD neurotoxin 6-OHDA will be compared to TNF-α (10 ng/ml) or LPS (100 ng/ml), two known cytokines involved in neuroinflammatory processes and astrocytic activation.

Sister cultures will be used in time-course studies for detailed characterization of cellular functions (neuron viability, astrocytes proliferation, mitochondrial function, apoptosis, etc.) by using Operetta CLS™, which enables to perform bright field and fluorescence imaging on a large number of samples using specific dyes and probes. We will assess NADP+/NADPH, GSH/GSSG and cysteine/cystine balance in CTR and 6-OHDA treated cultures, as well as following stimulation with TNF-α (10 ng/ml) or LPS (100 ng/ml). In addition, we will assess mitochondrial bioenergetics using the Seahorse Extracellular Flux Analyser.

Together with the metabolic profiling, these studies will allow to: 1) understand mechanisms interconnecting subcellular, compartmentalized NAD(H)/NADP(H) pool and how they regulate cellular redox states and cellular metabolism; 2) how NAD(H)- and NADP(H)-utilizing enzymes interact with signaling pathways linked to 6-OHDA or cytokines induced stress and energy metabolism; 3) Glutamate/glutamine and cysteine/cysteine metabolism and their correlation with the redox system.

Feasibility and financial support

Mice are maintained in our animal facility, thus the cost will be contained. Most of the reagents (antibodies for IHC, specific dyes for mitochondrial and cellular function and plasticware for the first year) are already available in the lab. There is possibility to obtain additional funds for the following years.

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