Carbon monoxide as a neuroprotective agent against hypoxia-ischemia and reperfusion: apoptosis and autophagy targeting

Supervisor: Helena L.A. Vieira (hvieira@itqb.unl.pt)

Research Plan

State of the Art
Hypoxia-ischemia and reperfusion (HIR) is the main cause of brain damage leading to mortality and morbidity. In adults HIR is mainly due to stroke whether, in newborns and preterm infants, is caused by perinatal complications (Mattson and Kroemer 2003; Vannucci and Hagberg 2004). Low doses of carbon monoxide (CO) confer protection against apoptosis in several types of cells and tissues (Ryter et al. 2006; Bannenberg and Vieira 2009). Recently we have demonstrated that CO presents anti-apoptotic properties in neurons by inducing a preconditioning-like effect (Vieira et al. 2008). In astrocytes, carbon monoxide also prevents apoptosis by directly inhibiting mitochondrial membrane permeabilization, a key event during cell death (Queiroga et al. 2010; Queiroga et al. 2011). Moreover, in this system, reactive oxygen species and oxidized glutathione appear as signalling molecules, involved in preconditioning induction (Queiroga et al. 2010).

Autophagy is a process by which cytoplasmic components (macromolecules or organelles) are degraded by the lysosome. It was first recognized as a specific type of cell death, also called type 2. However, it is now increasingly accepted that cells may die coincidentally with autophagy rather than by autophagy. Furthermore, autophagy can be considered as a cytoprotective mechanism involved in cell adaptation to stress (Kroemer and Levine 2008). The autophagic process is associated with several physiological roles and pathological effects, including quality control of intracellular proteins and organelles, anti-aging, adaptive responses to starvation (the most described stimulus for autophagy), and suppression of tumour formation (Kroemer and Levine 2008). In cerebral HIR, autophagy is activated and participates in neuroprotection (Carloni et al. 2008).

Objectives
Our aim is to understand the cellular and biochemical pathways involved in CO-induced cytoprotection, in particular the crosstalk between apoptosis and autophagy. Still, it will be addressed whether and how CO modulates autophagy, controlling cell survival or death and the degradation of unwanted proteins or organelles.

Methodology
The role of CO in the modulation of autophagy will firstly be addressed by using in vitro primary cultures of astrocytes and neurons. The expression of autophagic related genes (such as Atg 5, Atg 7, Atg 10, Atg 12, Atg 16, Ampa and Beclin-1) will be traced by reverse-transcriptase quantitative PCR (mRNA synthesis) and western blots (protein expression). Autophagic flux will be followed by measuring the conversion between LC3 I into LC3 II, in the presence or absence of inhibitors of lysosomal activity. Moreover, chemical inhibitors or small interference RNA (siRNA) transfection will prevent autophagic processes, and cell death
prevention by CO will be re-evaluated in order to assess the cytoprotective role of CO-induced autophagy.

We will address the associated cellular and biochemical mechanisms, as well as the cross-talk pathways between autophagy and cell death control (apoptosis and necrosis). Some of our targets for the study follow: (i) the complex Beclin-1 and Bcl-2 (anti-apoptotic protein); (ii) BH3-only pro-apoptotic proteins BNIP3 and BNIP3L, known to be associated with hypoxia-induced autophagy and (iii) calpain (classically associated with necrosis) which is involved in lysosomal permeabilization and activity. The master student will be directly involved in: (i) isolating and maintaining primary cell cultures; (ii) silencing gene expression in these cells; (iii) assessing gene expression and (iv) studying apoptotic and autophagic cell processes. Flow cytometry, fluorescent microscopy, western blots and RT-Q-PCR are other essential tools that will be used in this research project.

Local
CEDOC@IGC, Faculdade de Ciências Médicas, UNL, Laboratório Associado de Oeiras
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Scientific Collaborations directly involved in this project
- Dr Patricia Boya, Centro de Investigaciones Biológicas, CIB-CSIC, SPAIN – expert in autophagy
- Prof Catherine Brenner-Jan, Faculté de Pharmacie, Université Paris 11, France – expert in mitochondrial control of apoptosis

References