



## Seminarios internos del IBFG

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### Protein stability mediated by calpains during neuronal ischemic tolerance

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**Salón de actos del IBFG**

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Since the mid-1990s, there have been increasing studies to identify the mechanisms involved in the brain's ability to protect itself from Ischemia, known as endogenous neuroprotection. During these years a better understanding of the biochemical processes underlying the complex ischemic cascade was gained. Numerous studies suggested that subtoxic stimulation activating the cell signaling pathways, known as preconditioning (PC), promotes the reduction of brain damage and thus ischemic tolerance (IT) in cases of greater ischemic injury.

This effect of PC in the human brain was further supported in clinical trials. Recently, our research group was able to show that ischemic stroke patients who had previously suffered a transient ischemic attack showed a better prognosis compared to those that had not (1). Moreover, we have previously demonstrated that PC based on subtoxic stimulation of glutamate NMDA receptors increases the level of protein E3-ubiquitin ligase (MDM2) in primary cultured cortical neurons. These PC-increased MDM2 protein levels promotes its interaction with p53 and its subsequent destabilization, thus inhibiting the p53/PUMA/caspase-3 apoptotic pathway, and inducing neuronal tolerance to a prolonged ischemic stimulus (2).

Given the importance of protein stability associated with PC-induced neuronal survival, we have suggested to study another cellular systems controlling protein stability/degradation, the cysteine proteases, called calpains (CAPN). More specifically, two well-known CAPN isoforms,  $\mu$ -calpain (CAPN1) and m-calpain (CAPN2), which have been associated with the development of neurodegenerative diseases such as Alzheimer's. The main objective of our study, therefore, was to identify the proteolytic function of these two CAPN-isoforms during PC-induced neuronal tolerance and in response to ischemia. We were able to show that in primary neuronal cultures the PC model, developed to expose a short time of ischemia, inhibits the activation of Caspase-3 and neuronal apoptosis caused by prolonged ischemia. In addition, the in vivo PC model reveals a reduced volume of infarction in preconditioned animals, compared to non-conditioned animals and in response to ischemic damage. The PC-associated ischemic tolerance appears to be mediated by changes in the expression, activity and sublocation of CAPN2 in cortical neurons in response to ischemic damage. Finally, our study demonstrates that PC promoted changes in the calpain-mediated proteolysis of fodrin, one of the proteins responsible for the maintenance of the cellular cytoskeleton, both in vitro and in vivo. Fodrin also is highly involved in the development of several diseases associated with other biological functions, such as molecule transport, cell migration, genomic integrity and apoptosis. Thus, these preliminary results suggest that CAPN2 could play an important role during

PC-induced neuronal IT, through the regulation of fodrin, which would be a highly relevant mechanism in the neuronal response to ischemic damage.

(1). *The neuronal ischemic tolerance is conditioned by the Tp53 Arg72Pro polymorphism.* Ramos-Araque ME, Rodriguez C, Vecino R, Cortijo Garcia E, de Lera Alfonso M, Sanchez Barba M, Colàs-Campàs L, Purroy F, Arenillas JF, Almeida A, Delgado-Esteban M. *Transl Stroke Res.* 2019 Apr;10(2):204-215.

(2). *The MDM2-p53 pathway is involved in preconditioning-induced neuronal tolerance to ischemia.* Vecino R, Burguete MC, Jover-Mengual T, Agulla J, Bobo-Jiménez V, Salom JB, Almeida A, Delgado-Esteban M. *Sci Rep.* 2018 Jan 25;8(1):1610.