

● PERSPECTIVE

Neuroprotection by salubrinal treatment in global cerebral ischemia

Most common cerebrovascular accidents (CVAs) result from the occlusion of a blood vessel and are called ischemic stroke. The interruption of the blood flow reduces the oxygen and glucose levels in the neural parenchyma, which in turn decreases the energy of the cells and compromises their homeostasis. This condition has to be reverted for cell survival. One of the homeostatic processes impaired by cerebral ischemia is protein folding in the endoplasmic reticulum (ER) lumen where unfolded proteins are accumulated (Degracia and Monti, 2004). This situation, known as ER stress, elicits the so called unfolded protein response (UPR), whereby the cell tries to restore homeostasis to prevent its death. ER stress activates protein sensors that trigger the three different pathways described so far for UPR. Early UPR signaling events are initiated by the protein kinase RNA-like endoplasmic reticulum kinase (PERK). This UPR pathway is characterized by the phosphorylation of the eukaryotic translation initiation factor 2 α (eIF2 α), which blocks most of protein translation and activates the transcription factor 4 (ATF4). This factor regulates the activation of genes that protect the cell against metabolic consequences of ER stress. PERK-ATF4 UPR pathway activation enhances the protein folding capacity of the ER and is essential for reducing ER stress. This pathway is turned off by protein phosphatase 1 (PP1) which dephosphorylates p-eIF2 α (reviewed by Godin et al., 2016). The use of the PP1 blocker, salubrinal (Sal), which is able to penetrate into brain tissue *in vivo* (Sokka et al., 2007), provides a mechanism to maintain activated the PERK-UPR pathway (Ohri et al., 2013).

Inhibition of ER stress has been reported to reduce brain damage from ischemia/reperfusion (I/R) injury and represents a putative therapeutic strategy to alleviate the effects of stroke. The first report on the effects of Sal on ischemia was reported in a middle cerebral artery occlusion (MCAO) model. In this study, the intraperitoneal administration of Sal 30 minutes before MCAO decreased the infarct volume and an increased eIF2 α phosphorylation in the following hours after the ischemic insult (Nakka et al., 2010). Post-ischemic treatment with salubrinal also provided neuroprotection in a two vessel (2VO) occlusion/hypotension rat model of global cerebral ischemia (GCI). In this case, Sal was also administered intraperitoneally but 1 hour following ischemia. This study compares the responses in the Cornu Ammonis 1 (CA1) hippocampal area, widely reported to be more sensitive to the ischemic damage, with the less sensitive CA3 hippocampal area and cerebral cortex (Anuncibay-Soto et al., 2016). There are some differences between these models. MCAO presents an ischemic core characterized by severe ischemia (blood flow below 10% to 25% compared to normal values). This area presents a rapid depletion of the energy stores which leads to necrosis of neurons and supporting glial cells. Areas that surround the ischemic core (penumbra) present mild to moderate ischemia and may remain viable if the blood flow is restored. An ischemic core is not observed in the GCI model as the interruption of the blood flow for 15 minutes to all the brain turns it into a global penumbral zone. However, this blocking does not result in a similar effect within distinct regions of the brain, and differences in ischemic-dependent ER stress can be observed, as described between cerebral cortex and hippocampus (Llorente et al., 2013). These differences would depend on the ischemic

vulnerability of each region instead of the time-dependent progressive damage that irradiates from the ischemic core in the MCAO model. Differences in restoring the normal blood flow in distinct areas of the brain could also contribute to explain different ischemia-dependent damage in the GCI model.

Neuroprotective effect of preventing ER stress: Salubrinal has been described as a neuroprotective agent in different nervous system pathologies including brain ischemia, showing the relevance of decreasing ER stress as a therapeutic target to alleviate neural damage. Thus, treatment with salubrinal provides cytoprotection associated to the enhanced PERK-eIF2 α signaling in spinal cord injury (Sokka et al., 2007; Ohri et al., 2013). In traumatic brain injury, alleviation of ER stress by salubrinal treatment prevents neuronal demise in the cortex of injured mice (Rubovitch et al., 2015). As mentioned above, the neuroprotective effect of salubrinal on ischemic damage has been reported both in MCAO and in GCI. MCAO damage is usually estimated as a measure of the infarct volume. In contrast, neuroprotective effect quantification in GCI needs to take into account cell mortality since there is no infarct volume to be measured. The neuroprotective effect of salubrinal in GCI has been demonstrated in CA1 7 days after injury as a reduction in neuronal loss (Anuncibay-Soto et al., 2016). This later report also hypothesizes that UPR in CA1 is not able to overcome the ER stress elicited by the ischemic insult and salubrinal provides additional strength to overcome the ER stress in this area.

Inflammation, blood-brain barrier (BBB) and ER stress: Neuroinflammation plays a dual role in the response to brain ischemic insult. The earlier phases of inflammation try to alleviate the neurodegenerative damage that appears after stroke. However, in later stages, inflammation becomes uncontrolled (sterile inflammation) and contributes to the extension of the injury (Ceulemans et al., 2010). One of the main consequences of inflammation after stroke is the impairment of the BBB. The resultant permeabilization allows the uncontrolled passage of cells, particles and large molecules from blood to the neural parenchyma altering its homeostasis and facilitating its invasion by potential harmful components. The BBB is usually considered to present the same characteristics along the brain. However, BBB properties along the nervous central system (CNS) could be dif-

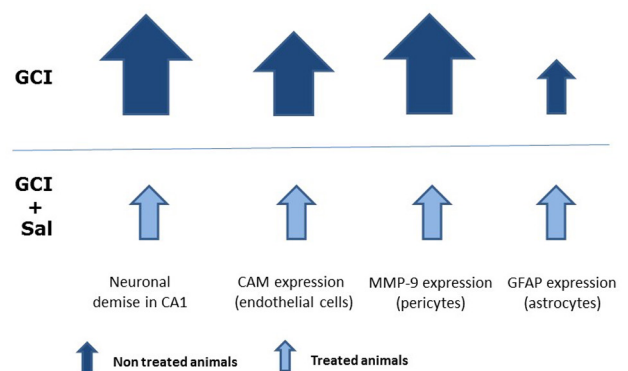


Figure 1 The main effects of post-ischemic treatment with salubrinal on the NVU components in a model of GCI.

Main cells modified by ER stress seem to be neurons, endothelial cells and pericytes, while astroglia remain almost unaffected. NVU: Neurovascular unit; GCI: global cerebral ischemia; CA1: Cornu Ammonis 1; CAM: cell adhesion molecule; MMP-9: matrix metalloproteinase 9; GFAP: glial fibrillary acidic protein.

ferent in the light of different inflammatory responses observed in cerebral cortex and hippocampal areas (Anuncibay-Soto et al., 2016). The idea of heterogeneity in BBB properties is also supported by regional differences induced by ischemia and age on the cell adhesion molecules (CAMs) of endothelial cells in a GCI model (Anuncibay-Soto et al., 2014).

In the last years, the BBB has been considered as a neurovascular unit (NVU) which includes vascular cells (pericytes, vascular smooth muscle cells, endothelial cells), glial cells (astrocytes, microglia, oligodendrocytes) and neurons. The NVU concept integrates the intercellular crosstalk that regulates the blood flow in brain and the integrity in the BBB. New therapeutic strategies to repair and restore brain function after injury have been proposed to focus on the role of the NVU components in protection and repair, rather than their role in cerebral blood flow regulation (Quillinan et al., 2016). For example, endothelial cells in the impaired NVU promote the recruitment of leukocytes and their migration through the BBB into the neural parenchyma leading to the breakdown of the immunoprivilege properties of SNC.

Few studies have addressed the role of ER stress in NVU components. In spinal cord injury, salubrial treatment has been shown to increase the transcript levels of oligodendrocyte and neuron markers, but the effect seems to be irrelevant in astrocytes (Ohri et al., 2013). Reduced effects of salubrial on astrocyte ER stress have also been indicated in a GCI model (Anuncibay-Soto, 2016). In contrast, ER stress in endothelial cells has been reported to play a crucial role in the mechanisms of adhesion and the subsequent leukocyte transmigration through the blood retinal barrier (BRB) (Li et al., 2011) as well as in the BBB integrity (Anuncibay-Soto et al., 2016). Both studies describe the interaction of ER stress with the response to the tumor necrosis factor alpha (TNF-alpha), one of the most prominent cytokines involved in the activation of inflammation. Both reports conclude that ER stress results in the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling, a crucial transcript factor for cell survival. These studies also report the role of ER stress in the expression of the adhesion molecules ICAM-1 and VCAM-1. These CAMs are responsible for adhesion and migration of leukocytes, a critical mechanism in the maintenance of the integrity in both the BRB and the BBB. In the GCI model, it has also been reported that the treatment with salubrial decreases the matrix metalloproteinase 9 (MMP-9), a widely accepted marker of the integrity of the BBB. It is assumed that pericytes are strongly modified by ER stress since MMP-9 is mainly produced by this cell type (Anuncibay-Soto et al., 2016).

Thus, the treatment with salubrial evidences that mitigation of ER stress by UPR plays a pivotal neuroprotective role in ischemia. This alleviation seems to modify the inflammatory response but it does not affect in the same way the different NVU components. Thus, endothelial cells, pericytes and neurons seem to present a stronger response to the post-ischemic salubrial treatment (**Figure 1**). This represents novel opportunities for the development of new anti-stroke therapies. The studies based on salubrial treatment also support the idea of local differences in the BBB properties. Moreover, they have led to the hypothesis that the enhancement of the homeostatic response in some regions with a limited capacity of response could make them less vulnerable to ischemia. Finally, the effect of salubrial seems to be limited to the first stages of ischemia, but could be used in a combined therapy with other therapeutic agents. Therefore, salubrial emerges as a powerful tool in the study of neurodegenerative mechanisms.

We want to thank Diego Pérez Rodríguez, Enrique Font and Marta Regueiro-Purriños for scientific collaboration in the CGI salubrial study. We apologize to all authors whose work was not cited here due to restrictions of the paper length. This study was supported by MINECO and FEDER funds, references BIO2013-49006-C2-2-R, that supports BAS, and RTC-2015-4094-1, that supports M SG.

Berta Anuncibay-Soto, María Santos-Galdiano,
Arsenio Fernández-López*

Area de Biología Celular, Instituto de Biomedicina, Universidad de León, León, Spain

*Correspondence to: Arsenio Fernández-López, Ph.D.,
aferl@unileon.es.

Accepted: 2016-11-04

orcid: 0000-0001-5557-2741 (Arsenio Fernández-López)

doi: 10.4103/1673-5374.194711

How to cite this article: Anuncibay-Soto B, Santos-Galdiano M, Fernández-López A (2016) Neuroprotection by salubrial treatment in global cerebral ischemia. *Neural Regen Res* 11(11):1744-1745.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

References

- Anuncibay-Soto B, Pérez-Rodríguez D, Llorente IL, Regueiro-Purriños M, Gonzalo-Orden JM, Fernández-López A (2014) Age-dependent modifications in vascular adhesion molecules and apoptosis after 48-h reperfusion in a rat global cerebral ischemia model. *Age (Dordr)* 36: 9703.
- Anuncibay-Soto B, Pérez-Rodríguez D, Santos-Galdiano M, Font E, Regueiro-Purriños M, Fernández-López A (2016) Post-ischemic salubrial treatment results in a neuroprotective role in global cerebral ischemia. *J Neurochem* 138:295-306.
- Ceulemans AG, Zgavc T, Kooijman R, Hachimi-Idrissi S, Sarre S, Michotte Y (2010) The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia. *J Neuroinflammation* 7:74.
- DeGracia DJ, Montie HL (2004) Cerebral ischemia and the unfolded protein response. *J Neurochem* 91:1-8.
- Godin JD, Creppe C, Laguesse S, Nguyen L (2016) Emerging roles for the unfolded protein response in the developing nervous system. *Trends Neurosci* 39:394-404.
- Li J, Wang JJ, Zhang SX (2011) Preconditioning with endoplasmic reticulum stress mitigates retinal endothelial inflammation via activation of X-box binding protein 1. *J Biol Chem* 286:4912-4921.
- Llorente IL, Burgin TC, Pérez-Rodríguez D, Martínez-Villayandre B, Pérez-García CC, Fernández-López A (2013) Unfolded protein response to global ischemia following 48 h of reperfusion in the rat brain: the effect of age and meloxicam. *J Neurochem* 127:701-710.
- Nakka VP, Gusain A, Raghuram R (2010) Endoplasmic reticulum stress plays critical role in brain damage after cerebral ischemia/reperfusion in rats. *Neurotox Res* 17:189-202.
- Ohri SS, Hetman M, Whittemore SR (2013) Restoring endoplasmic reticulum homeostasis improves functional recovery after spinal cord injury. *Neurobiol Dis* 58:29-37.
- Quillinan N, Herson PS, Traystman RJ (2016) Neuropathophysiology of brain injury. *Anesthesiol Clin* 34:453-464.
- Rubovitch V, Barak S, Rachmany L, Goldstein RB, Zilberstein Y, Pick CG (2015) The neuroprotective effect of salubrial in a mouse model of traumatic brain injury. *Neuromolecular Med* 17:58-70.
- Sokka AL, Putkonen N, Mudo G, Pryazhnikov E, Reijonen S, Khiroug L, Bel-luardo N, Lindholm D, Korhonen L (2007) Endoplasmic reticulum stress inhibition protects against excitotoxic neuronal injury in the rat brain. *J Neurosci* 27:901-908.