PERSPECTIVE

All roads go to Salubrinal: endoplasmic reticulum stress, neuroprotection and glial scar formation

Central nervous system (CNS) injuries caused by cerebrovascular pathologies (*e.g.*, stroke) or mechanical contusions (*e.g.*, traumatic brain injury) disrupt the blood-brain barrier (BBB) that protects the CNS microenvironment from a direct contact with blood substances and cells. The initial neural damage caused by the trauma and the ischemic process is extended in time by a secondary neuronal loss due to the reactive microglial cells and blood leukocytes that migrate to the lesion site and produce inflammatory mediators (*e.g.*, reactive oxygen species) that increase cell death. The severity of the neural damage in patients will determine the extension of the short- and long-term physical, cognitive and emotional impairments associated with these pathologies (McAllister, 2011).

Glial cells (mainly astrocytes) and profibrotic mesenchymal cells (meningeal fibroblasts, perivascular fibroblasts and pericytes) react to the injury and migrate to the lesion site, secreting extracellular matrix proteins and inducing a new *glia limitans* called glial scar (Fawcett and Asher, 1999). This physical structure reduces the leakage of blood substances and the migration of blood cells to the lesion site, reducing cell death and facilitating the recovery of tissue homeostasis (Raposo and Schwartz, 2014). However, the glial scar is one of the main obstacles to axonal regeneration after injury (Fawcett and Asher, 1999).

Secretory and transmembrane proteins are synthesized in the ribosomes coupled to the endoplasmic reticulum (ER). These proteins are folded by ER-resident chaperones that ensure a proper transport from ER to the Golgi apparatus. There, a quality control mechanism recognizes misfolded and/or unfolded proteins and induces their degradation in the proteasome, avoiding their accumulation in the lumen of the ER. Diverse pathological conditions (*e.g.*, ischemia, trauma, viral and bacterial infections) may induce the accumulation of misfolded and/or unfolded proteins in the ER that trigger ER stress response. If this response cannot restore homeostasis, it may become chronic, resulting in cell death (Hetz and Mollereau, 2014).

Salubrinal is a small molecule with cytoprotective effect on ER stress-induced cell death (Boyce et al., 2005). The neuroprotective effect of Salubrinal has been reported in an excitotoxic neuronal injury model in rat brain (Sokka et al., 2007), in a mouse model of sleep apnea (Zhu et al., 2008), in a cerebral ischemia/reperfusion injury model in rats (Nakka et al., 2010) and traumatic brain injury model in mice (Rubovitch et al., 2015). Moreover, Salubrinal had a cytoprotective effect on oligodendrocytes, reducing demyelination and improving functional recovery after spinal cord injury in mice (Ohri et al., 2013). Salubrinal treatment reduces cell death through the diminution of the ER stress response induced in CNS injury models (Sokka et al., 2007; Ohri et al., 2013), probably reducing ER protein overload in pathological conditions.

The phosphorylation status of the translational initiator eIF2α regulates protein translation in the ER. eIF2α is phosphorylated by four different kinases: GCN2 (activated by amino acid starvation), HRI (activated by heme deprivation, as well as by osmotic and heat shocks), PKR (activated by viral infections, some cytokines and growth factors) and PERK (activated by ER stress and hypoxia). Increasing the phosphorylation status of eIF2α attenuates the translation of secretory proteins that are synthesized in the ER. Conversely, reducing the phosphorylation of eIF2α increases the translation of secretory proteins. PP1α phosphatase forms a complex with GADD34 or CReP protein that dephosphorylates eIF2α. Salubrinal is an inhibitor of the protein phosphatase PP1 that attenuates the translation of secretory proteins, maintaining eIF2α highly phosphorylated (Boyce et al., 2005).

After CNS injury, reactive astrocytes express and secrete chondrotin sulfate proteoglycans (CSPGs), such as brevican, neurocan, versican and phosphacan, major axon growth inhibitory components of the glial scar (Fawcett and Asher, 1999). CSPGs consist of a large variety of core proteins, covalently linked to chondroitin sulfate glycosaminoglycans, synthesized in the ER and glycosylated in the Golgi apparatus. Both protein and glycosylated core of CSPGs have been described as axon growth inhibitors (Fawcett and Asher, 1999).

Glial scar formation is regulated by cytokines and growth factors released from platelets, blood cells and CNS endogenous cells that initially respond to the lesion and then to the subsequent inflammation. Growth factors such as epidermal growth factor (EGF), transforming growth factor β (TGFβ) and connective tissue growth factor (CTGF), and cytokines such as interleukin-6 (IL-6), interferon gamma (IFNγ), and IL-1β, regulate the expression and secretion of CSPGs by astrocytes (Asher et al., 2000).

Because astrocytes are the main producers of CSPGs and other profibrotic substances that form the glial scar, we studied in these cells the effect of Salubrinal on the expression and secretion of CSPGs (Barreda-Manso et al., 2015). Translational attenuation induced by Salubrinal (maintaining eIF2α phosphorylated) reduced the expression and secretion of CSPGs and other profibrotic proteins such as CTGF. Additionally, Salubrinal reduced the mRNAs for CSPGs and CTGF. These data suggests that Salubrinal might induce the degradation of non-translated ER-targeted protein mRNAs. This process will collaborate with the translational attenuation, to reduce protein overload in the ER.

We used an *in vitro* model of glial scar to determine whether Salubrinal may have a beneficial effect on neurite outgrowth from cortical neurons. A coculture of astrocytes and fibroblasts was treated with the profibrotic growth factor TGFβ. Cortical neurons grown on top of astrocytes-fibroblasts cocultures, treated with TGFβ showed a reduced length of their neurites compared to control cocultures.

However, pretreatment of the cocultures with Salubrinal, reverted the neurite outgrowth inhibition compared to cocultures treated with TGFβ only (Barreda-Manso et al., 2015). Although these data are preliminary and the effect of Salubrinal must be tested in an animal model of CNS injury before any conclusion, the data open the possibility of modulating extracellular matrix deposition and glial scar formation. As previously described, the glial scar is beneficial, because it reduces the leakage of blood substances and cells, helping to restore homeostasis in the injured tissue (Raposo and Schwartz, 2014). At this point, the question is, how much glial scar reduction is necessary to permit axonal regeneration and at the same time prevent leakage of blood content to the neural parenchyma? What dosage of Salubrinal and for how long the animals should be treated to have a beneficial effect?

Most of the articles that studied the effect of Salubrinal on diverse animal models of CNS injury, treated the animals for the first three days after the injury with a concentration of Salubrinal ranging from 1 to 5 mg/kg (Sokka et al., 2007; Zhu et al., 2008; Ohri et al., 2013; Rubovitch et al., 2015). Only one study has followed-up the animals longer than 3 days after the injury (Ohri et al., 2013), with an open field BMS locomotor analysis, performed weekly. They found that animals treated with Salubrinal after a spinal cord injury showed significantly higher functional recovery than untreated injured animals (Ohri et al., 2013). The authors presented BMS scores until 7 weeks after spinal cord injury. The animals treated with Salubrinal for the first 3 days postlesion (acute treatment) had better outcome than injured untreated animals. These data suggest that acute Salubrinal treatment at the concentration tested may not increase leakage of blood content to the neural parenchyma and it may not impede glial scar formation.

Obviously, more work is necessary before reaching further conclusions on the therapeutical effect of Salubrinal. However, the promising results of this drug deserve a careful trial.

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