

● PERSPECTIVE

Attenuation of endoplasmic reticulum stress as a treatment strategy against ischemia/reperfusion injury

Brain ischemic stroke is the leading cause of long-lasting injury, disability, and death in adults. Although the brain represents only about 2% of the total body mass, it consumes almost 20% of the body's oxygen. As a result, brain cells are extremely sensitive to hypoxia. Once cerebral ischemia occurs, the core of the infarct shows great rapidly necrotic cell death due to complete and prolonged oxygen and glucose deprivation. In contrast, surrounding this necrotic core is a region called penumbra, where neurons are partially perfused from collateral vessels. If sufficient circulation cannot be restored within a short time, these cells will gradually fail resulting in subsequent cell death by apoptosis. This implies the prompt restoration of blood flow to a penumbra area may allow threatened tissue to be salvaged. Therefore, rapid administration of thrombolytic agents such as heparin or tissue-type plasminogen activator (tPA) is used as a bolus treatment. Although the circulation is restored upon reperfusion, a surge of oxygen levels may cause an additional damage known as ischemia/reperfusion (I/R) injury (Liu et al., 2014). It is known I/R can cause oxidative damage, which triggers stress signaling eventually resulting in significant apoptotic death of neurons. There are a number of pathways involved in the I/R response. Particularly, the Akt pathway has been a great focus in preventing I/R-induced apoptosis. Akt is involved in cellular survival pathways through activation of neurotrophic signaling, resulting in inhibition of glycogen synthase kinase 3 β (GSK3 β) activity. GSK3 β is a serine-threonine kinase firstly discovered to phosphorylate and inactivate glycogen synthase, an enzyme in the glycogen synthesis pathway. Normally, activated Akt suppresses GSK3 β ; however, GSK3 β can be activated by I/R-induced injury, which contributes the mitochondrial dysfunction and subsequently induces neuronal apoptosis (Hanumanthappa et al., 2014). Thus, inhibition of GSK3 β is proposed as a putative therapeutic strategy after I/R. These mechanisms activated in the penumbra area may be responsible for the final fate of neurons and the degree of neurological damage in ischemic patients. Although GSK3 β inhibition is a rational strategy to combat ischemic stroke, the adverse events and a rather small therapeutic window limit its therapeutic success (Pradeep et al., 2014).

To overcome these challenges, targeting the alternative pathway may provide a better therapeutic effectiveness in ischemic stroke. Increasing evidence shows that endoplasmic reticulum (ER) stress may play a role in I/R-induced cell death. During ischemia, oxygen and glucose deprivation causes abnormalities in protein-folding processes and leads to accumulation of unfolded proteins in the ER, triggering an evolutionarily conserved response termed as unfolded protein response (UPR). This is activated by three transmembrane proteins including activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and protein kinase RNA-like endoplasmic reticulum kinase (PERK), which together induce both transcriptional and translational regulation of genes that provides an adaptive mechanism to cellular perturbations. To avoid the excessive accumulation of unfolded proteins, the protein kinase PERK phosphorylates translation initiation factor 2 (eIF2), resulting in global inhibition of protein synthesis to allow time to fix the

damage. Although the role of ER stress in neuronal ischemia remains undetermined, it is possible that activation of ER stress response could protect cells from proteotoxic stress imposed by rapid formation of misfolded protein aggregates (DeGracia and Montie, 2004). However, there is direct evidence that excessive or prolonged activation of ER stress can exacerbate ischemia injury. Particularly, the overactivation of the UPR may play a significant role in reperfusion, resulting in promoting cell death usually in the form of apoptosis (Roussel et al., 2013). It is known that reperfusion causes excessive UPR, triggering depletion of ER Ca²⁺, abnormal aggregation of proteins, impaired protein degradation, and in turn reduces *de novo* synthesis of survival-mediating proteins. Moreover, reperfusion also triggers reactive oxygen species (ROS) production and oxidative stress, resulting in protein misfolding and further induces or exacerbates UPR-induced apoptosis. These observations indicate that the prolonged ER stress is associated with brain I/R by continue activation of UPR. Therefore, modulation of abnormal UPR is thought as a potential therapeutic target in I/R-induced neuronal death.

Neuronal survival is tightly regulated by several neurotrophic factors (Maiese, 2015). Among these factors, the insulin/insulin like growth factor (IGF) signaling pathway plays a major role in response to ischemic stress. Upon activation by ligand binding, it triggers the intracellular activation of phosphoinositide 3-kinase (PI3K) and Akt. Akt has been widely reported to be one of the most important protein in promoting neuronal survival after cerebral ischemic insults (Mullonkal and Toledo-Pereyra, 2007). Activated Akt mediates several downstream responses, including cell survival, axonal growth, regeneration and protein synthesis enhancement through activation of mammalian target of rapamycin (mTOR). Akt pathway is also required for hypoxia-induced expression of hypoxia inducible factor-1 α (HIF-1 α), which has been identified as an important novel target in apoptosis resistance. In addition, activated Akt can phosphorylate GSK3 β at serine⁹ and inactivate this enzyme, this preventing GSK3 β from initiating a neuronal apoptotic pathway. Phosphorylation of Bad at serine¹³⁶ by Akt can also result in the inactivation of Bad and prevent neuronal apoptosis. Together, these studies suggest that Akt activity plays a crucial role in I/R-induced apoptosis. However, reoxygenation-sustained ER stress impairs survival signaling by reducing protein *de novo* synthesis. As a result, sustained activation of UPR during reoxygenation induces a reduction of Akt protein, thereby in turn promotes apoptosis. This indicates Inhibition of the excessive UPR response may be necessary for the preservation of neuronal function after I/R.

Based on these viewpoints, our recent results showed that the alleviation of ER stress by chemical chaperons such as 4-phenylbutyric acid (4-PBA) can provide neuroprotection during I/R. 4-PBA is a low molecular weight fatty acid that has been approved for clinical use as an ammonia scavenger. However, 4-PBA can also act as a chemical chaperone to alleviate ER stress by reducing the load of misfolded proteins retained in the ER. This indicates the use of 4-PBA may be a therapeutic approach for blocking the ER stress-induced neuronal death by I/R. As shown in **Figure 1**, we demonstrated that the upregulation of Akt works cooperatively with PI3K-Akt stimulator or GSK3 β inhibitor to promote cell survival during hypoxia/reoxygenation (Tung et al., 2015). This result is consistent with our results in which ER stress inhibitor salubrinal displays neuroprotective effects during I/R insults. Thus, the suppression of ER stress-mediated signaling may be a neuroprotective strategy to protect I/R-induced insults. This finding supports the idea that Akt is a major switch involved in regulating hypoxia-induced

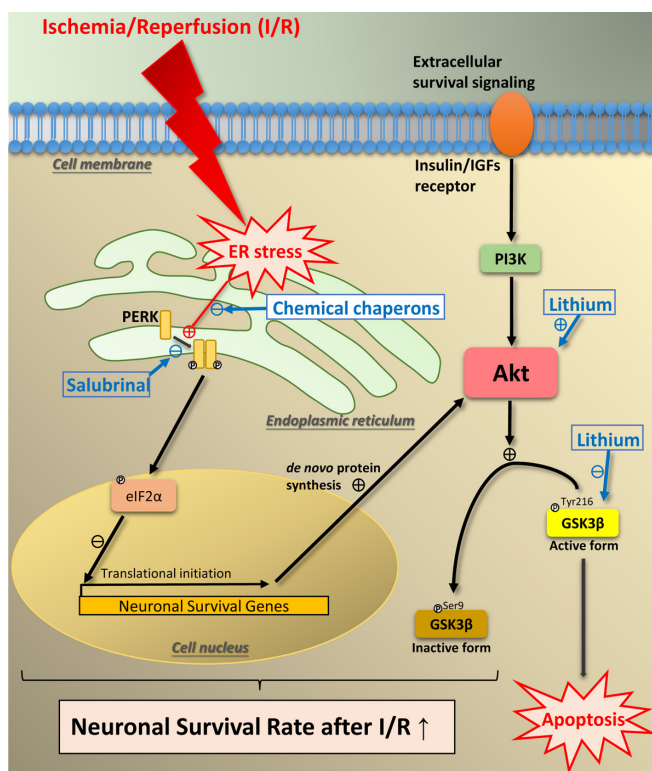


Figure 1 Schematic depiction of hypoxia/reoxygenation-induced endoplasmic reticulum (ER) stress, and pharmacologic strategies to protect neurons from hypoxia/reoxygenation injury.

During hypoxia/reoxygenation, oxygen and glucose deprivation causes a significant lack of energy, which induces a defect in protein-folding processes and triggers ER stress responses. These activities activate protein kinase RNA-like endoplasmic reticulum kinase (PERK) and subsequent phosphorylates translation initiation factor 2 (eIF2), resulting in global inhibition of survival protein synthesis such as Akt. The reduced levels of Akt diminish extracellular survival signaling, resulting in the failure of glycogen synthase kinase 3 β (GSK3 β) inhibition, which potentiates downstream apoptotic signaling and induces cell death. On the contrary, therapeutic candidates against ER stress may display neuroprotective effects by rescuing suppressed Akt biosynthesis, which can work cooperatively with compounds promoted survival signaling thereby attenuating hypoxia/reoxygenation-induced neuronal cell death.

cell death, and inhibition of ER stress is beneficial to ischemia-induced cellular damage during reoxygenation. Similarly, there is increasing evidence that Akt plays a crucial role in ER stress-elicited cell dysfunction. The mechanism of the loss of Akt proteins in late hypoxia is still not fully elucidated, although it appears to be a result of protein degradation by a non-proteasomal dependent pathway such as autophagy (Box et al., 2010). Autophagy is an evolutionally conserved process involved in the quality control mechanism to maintain homeostasis of the cell. Although autophagy has been suggested to promote cell survival under stress, excessive autophagy have also been associated with various forms of cell death. This indicates an essential role of autophagy regulation in I/R-induced neuronal cell death. It is known several kinases, particularly mammalian target of rapamycin (mTOR) and Akt can regulate autophagy process. Once activated, Akt phosphorylates and inhibits tuberous sclerosis complex-2 (TSC2) that activates mTOR and thereby reduces autophagy. Therefore, activation of Akt-mTOR signaling can

inhibit ER stress-induced autophagy. This finding is consistent with our previous study that has demonstrated the activation of the Akt pathway by insulin-related neurotrophic factors is a protective event during amyloid β (A β) exposure, an aggregated peptide-mediated apoptosis involved in ER stress-induced neurotoxicity (Kornelius et al., 2015). This implies the upregulation of Akt signaling may be a possible neuroprotective strategy for ameliorating ER stress-induced neuronal cell death.

Neurons are thought to be sensitive to proteotoxicity, and there are many reports that ER stress is involved in several neurological diseases, including I/R injury, type 2 diabetes, and some neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. This makes ER stress can be recognized as a probable instigator of pathological cell death and dysfunction. As a result, mediators of ER-initiated cell death are therapeutic candidates against cell death by ER stress. In conclusion, our recent findings provide a possible mechanism about how ER stress mediates their neurotoxic effects, and it is possible that pharmacologically, such as in the case of ER stress inhibitors might be used to develop a novel strategy for I/R-induced damage.

Chih-Li Lin*

Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, China

*Correspondence to: Chih-Li Lin, Ph.D., dll@csmu.edu.tw.

Accepted: 2015-09-28

orcid: 0000-0003-4553-3727 (Chih-Li Lin)

doi: 10.4103/1673-5374.169615 <http://www.nrronline.org/>

Lin CL (2015) Attenuation of endoplasmic reticulum stress as a treatment strategy against ischemia/reperfusion injury. *Neural Regen Res* 10(12):1930-1931.

References

- Box AH, Kim SM, Demetrick DJ (2010) AKT loss in human epithelial cells treated with severe hypoxia. *Biochim Biophys Acta* 1803:951-959.
- DeGracia DJ, Montie HL (2004) Cerebral ischemia and the unfolded protein response. *J Neurochem* 91:1-8.
- Hanumanthappa P, Densi A, Krishnamurthy RG (2014) Glycogen synthase kinase-beta3 in ischemic neuronal death. *Curr Neurovasc Res* 11:271-278.
- Kornelius E, Lin CL, Chang HH, Li HH, Huang WN, Yang YS, Lu YL, Peng CH, Huang CN (2015) DPP-4 inhibitor linagliptin attenuates abeta-induced cytotoxicity through activation of AMPK in neuronal cells. *CNS Neurosci Ther* 21:549-557.
- Liu J, Li J, Yang Y, Wang X, Zhang Z, Zhang L (2014) Neuronal apoptosis in cerebral ischemia/reperfusion area following electrical stimulation of fastigial nucleus. *Neural Regen Res* 9:727-734.
- Maiese K (2015) Novel applications of trophic factors, Wnt and WISP for neuronal repair and regeneration in metabolic disease. *Neural Regen Res* 10:518-528.
- Mullonkal CJ, Toledo-Pereyra LH (2007) Akt in ischemia and reperfusion. *J Invest Surg* 20:195-203.
- Pradeep H, Densi A, Rajanikant GK (2014) Glycogen synthase kinase-3beta in ischemic neuronal death. *Curr Neurovasc Res* 11:271-278.
- Roussel BD, Kruppa AJ, Miranda E, Crowther DC, Lomas DA, Marciniak SJ (2013) Endoplasmic reticulum dysfunction in neurological disease. *Lancet Neurol* 12:105-118.
- Tung WF, Chen WJ, Hung HC, Liu GY, Tung JN, Huang CC, Lin CL (2015) 4-phenylbutyric acid (4-PBA) and lithium cooperatively attenuate cell death during oxygen-glucose deprivation (OGD) and reoxygenation. *Cell Mol Neurobiol* 35:849-859.