Review Article

Disparity among Neural Injury Models and the Unfolded Protein Response

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Abstract

Ischemic stroke and traumatic brain injury both cause endoplasmic reticulum stress and the unfolded protein response. Both injuries initially produce reactive oxygen species, increase intracellular calcium levels, and induce inflammation. The secondary effects from these brain injuries are similar and only differ in their timing and duration. Preventing the immediate effects of ischemic stroke or traumatic brain injury is challenging due to short onset of injury, but mitigating the secondary effects is a therapeutically targetable option. Preventative therapies using pharmacological agents have been utilized in pre-clinical models of neural injury to ameliorate secondary effects such as apoptosis and neurodegeneration. The connection between ER stress, apoptosis, and subsequent neurodegeneration has been proposed, but not yet causally linked. Researchers are now pursuing effective treatment strategies to suppress the secondary effects of neural injury in order to mitigate the development of chronic deficits. Preventative approaches that target secondary effects of brain injury and how these may correlate to better treatment options for patients.

ABBREVIATIONS

IS: Ischemic Stroke; TBI: Traumatic Brain Injury; ER: Endoplasmic Reticulum; UPR: Unfolded Protein Response; ROS: Reactive Oxygen Species; PERK: PKR-like ER Kinase; IRE1: Inositol Requiring Enzyme 1; ATF6: Activating Transcription Factor 6; BiP: Binding Immunoglobulin Protein; eIF2a: Eukaryotic Initiation Factor 2 Alpha; ATF4: Activating Transcription Factor 4; CHOP: C/EBP Homology Protein; GADD34: Growth Arrest And Damage Inducible Agent 34; PP1: Protein Phosphatase 1; XBP1: X-box binding protein 1; TRAF6: TNF Receptor-Associated Factor 6; Akt: Protein Kinase B; NF-kB: Nuclear Factor Kappa Beta; IKK: Inhibitors of Nuclear Factor Kappa B; Bcl-2: B Cell Lymphoma 2; ERAD: ER Associated Degradation; AD: Alzheimer’s Disease; DHA: Docosahexaenoic Acid

INTRODUCTION

Cellular responses occur rapidly following injury to the central nervous system. Cellular response mechanisms are triggered from a lack of oxygen (stroke) or from mechanical damage (trauma). Each injury type can invoke similar acute cellular responses; however, inaccurate overlapping of the secondary effects occurs between injury modalities. The secondary biochemical effects can be therapeutically targeted in order to provide patients with valuable treatment options to attenuate further injury. Endoplasmic reticulum (ER) stress has been implicated in a variety of neural injury models including ischemic stroke (IS) [1-3] and traumatic brain injury (TBI) [4-6]. Immediately following brain injury, excitatory glutamate release increases intracellular Ca2+ levels causing ER stress. ER stress then triggers the unfolded protein response (UPR) as an endogenous means of cellular repair. Apoptosis and neurodegenerative disease development following neural injury. In this review, we examine therapeutic approaches that target secondary effects of brain injury and how these may correlate to better treatment options for patients.

What is known is that neural injury causes massive neuronal depolarization and a resulting large influx of Ca2+. Increased intracellular Ca2+ can produce inflammation and reactive oxygen species (ROS) which are known to cause neuronal death and are...
associated with chronic disease pathology [9]. At the subcellular level, \( \text{Ca}^{2+} \) combines with calmodulin to activate the calpain protease that contributes to the progression of neural injury [10]. An important calpain substrate is p35, which is an activating cofactor for Cyclin dependent kinase 5 (Cdk5) [11]. Calpain-dependent cleavage of p35 produces the truncated protein p25. Cdk5/p25 stimulates abnormal activity and mediates neuronal cell death [12]. The neurofilament binding protein, Tau, is a substrate of Cdk5/p25 [13], and may contribute to the manifestation of neurodegeneration following neural injury. The hyperphosphorylation of tau, and the resulting formation of neurofibrillary tangles, may help characterize neural injury pathology [14]. A theoretical mechanistic diagram proposing a causal link between the UPR and apoptosis with subsequent neurodegeneration has been provided (Figure 1).

The main difference between the UPR after IS compared to TBI is the duration and severity of insult. IS invokes decoupling of oxidation to the brain and is an injury of long duration with multiple phases of inflammatory cascades. IS also diminishes glucose, or the brain’s energy supply, disrupting Ca\(^{2+}\) homeostasis. TBI on the other hand, occurs over a very short duration, and is more often a less severe injury that can even go undetected. The rotational and acceleration/deceleration components commonly tears axons apart leading to a robust gliosis response [15,16]. The injury can also cause \( \text{Ca}^{2+} \) perturbations much like I/R injury; however, the shock wave seems to harm the cell in an energy-independent process. The damage done by TBI makes membranes more permeable to \( \text{Ca}^{2+} \) and even to extracellular proteins [17]. In addition, enhanced glutamate-signaling causes a spike in intracellular \( \text{Ca}^{2+} \). The duration of injury as well as severity provides key targets for specific injury-type treatments.

**ENDOPLASMIC RETICULUM FUNCTION**

When the brain is deprived of oxygen from IS, or withstands physical injury from TBI, intracellular \( \text{Ca}^{2+} \) and reactive oxygen species (ROS) accumulate in the cytoplasm [18]. These events cause proteins to unfold, and when the ER becomes overwhelmed and struggles to re-fold the unfolded proteins, the UPR ensues [19]. In the short-term, the UPR can promote cell survival through three separate mechanisms: (1) attenuation of global translation, (2) upregulation of stress response genes, and (3) degradation of unfolded proteins [20]. However, when the response is prolonged, from a severe neural injury for example, neurons degrade [21], or commit to undergoing apoptosis [22]. Preserving the adaptive response to unfolded proteins while preventing subsequent apoptosis is a topic being heavily investigated in the field of neural injury.

The UPR begins when binding immunoglobulin protein (BIP) preferentially binds to unfolded proteins in the ER lumen exposing the three ER transmembrane proteins: (1) PKR-like ER kinase (PERK), (2) inositol requiring kinase 1 (IRE1), and (3) activating transcription factor 6 (ATF6) [23]. Each

![Figure 1 Schematic of the link between ER stress apoptosis and tau kinase activity.](Image)
adaptive arm of the UPR is triggered by Ca²⁺ perturbations and/or the overabundance of unfolded proteins in the ER lumen. Distinct downstream components of these arms have different characteristic responses to unfolded proteins [24]. Each pathway is different based on timing and duration, and may overlap with one another. In addition the UPR closely correlates with the intrinsic and extrinsic apoptotic pathways [25]. The next step in experimental research is to map out the interplay of these pathways following IS, as well as TBI, to determine how they contribute to chronic neurodegenerative diseases in humans.

**PERK pathway**

The ER manages unfolded proteins through nuclear-mediated initiation of global translation inhibition. When the unfolded proteins accumulate, the chaperone protein BiP, dissociates from the ER transmembrane protein PERK and preferentially binds to unfolded proteins [26]. BiP can restore protein conformation as a first line defense against protein misfolding [27]. Following BiP dissociation, PERK auto phosphorylates and activates the first arm of the UPR [28]. Activated PERK, phosphorylates eukaryotic initiation factor 2 alpha (eIF2α), which is a direct inhibitor of global translation [29]. Inhibiting protein synthesis reduces the number of proteins that must be folded in the ER, and therefore allows the ER to refold damaged proteins after a traumatic event.

Although global translation is inhibited through the phosphorylation of eIF2α, a subset of stress response genes become more actively transcribed [30]. The UPR upregulates the following stress response genes: activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), and growth arrest and DNA damage inducible agent 34 (GADD34)[31]. ATF4 contains three open reading frames, which only get transcribed after eIF2α has been phosphorylated [32]. Exactly how ATF4 is transcriptionally regulated is currently unknown; however, other well-known transcriptional regulators are being investigated.

Acutely, ATF4 can upregulate BiP and promote protein refolding and cell survival[33]. ATF4 also promotes the translation of GADD34, a regulatory subunit of the Protein Phosphatase 1 (PP1) complex. GADD34 is known to dephosphorylate eIF2α and acts in the pro-survival feedback mechanism [34]. When the UPR persists, the transcription factor ATF4 converts from being constitutively active to stress active, and upregulates CHOP, another transcriptional regulator of downstream apoptotic cascades[35-37]. CHOP actively inhibits the anti-apoptotic factor B-cell lymphoma 2 (Bcl-2) [38], and promotes ROS production [39]. The cells that survive the initial injury are those that we seek to protect from secondary injury mechanisms. Upregulating stress response genes that aid in protein refolding, while downregulating stress response genes that promote apoptosis and degeneration are both viable treatment options for patients subject to neurotrauma.

**IRE1 Pathway**

The second arm of the UPR involves the ER transmembrane component IRE1. BiP dissociates from IRE1 when unfolded proteins accumulate within the ER lumen, and IRE1 autophosphorylates itself [40]. IRE1 becomes an endonuclease for x-box binding protein 1 (XBP1) by splicing out a 26bp intron from unspliced XBP1. Spliced XBP1 translocates to the nucleus and regulates several cellular functions [41,42]. Another defense mechanism of the UPR is to simply degrade improperly folded proteins. This process occurs through a collection of proteins known as the ER-associated degradation (ERAD) machinery [19]. This mechanism works in conjunction with the proteasome to prevent aberrant protein accumulation and ultimately to prevent cell apoptosis [43]. Initially, the upregulation of ERAD components is protective to cells by helping to decrease the unfolded protein load; however, when their activity persists a neurodegenerative phenotype can manifest [44]. The cellular response to XBP1 is dependent on duration of activation much like ATF4. XBP1 can promote cell survival during conditions of oxygen deprivation [45], yet has detrimental responses in other forms of injury [46]. When the UPR persists the IRE1 arm can deactivate, and endonuclease activity ceases[40]. As a result, XBP1 is no longer spliced to become a potent transcription factor for pro-survival genes, including BiP [47]. Eventually the cell will undergo apoptosis if ER stress does not subside.

**ATF6 Pathway**

ATF6 is the first component of the third arm of the UPR. Once activated by BiP dissociation, ATF6 transits to the golgi apparatus, is cleaved by site 1 and 2 proteases, and translocates to the nucleus to upregulate pro-survival genes [48]. ATF6 is known to regulate transcription of XBP1 [49], a component of the second arm of the UPR. ATF6 may also play a role in inflammation and apoptosis through activation of the nuclear factor kappa B (NF-kB) pathway [50]. The ATF6 arm of the UPR remains largely unstudied and should be considered to play a vital role in cellular fate regulation. Determining the timing and duration of the different ER stress mechanisms as well as their complex interplay will allow for the development of preventative therapeutic options.

**Targeted approach**

Generally, ER stress is a protective compensatory process in the short-term, with persistent ER stress activation being more detrimental to cells over time. Focused therapy must target the underlying UPR mechanisms that mediate this temporal transition. Understanding the regulation of transcription factors: ATF4, XBP1, ATF6and NF-kB, is critical in designing successful therapeutic approaches. Briefly, ATF4 can be viewed as influencing protein reduction, XBP1 viewed as mediating protein clearance, ATF6 promoting protein refolding and NF-kB inducing protein production. These transcription factors can promote cell survival acutely, but can also contribute to cell death during chronic activation. The broad role of each transcription factor following both IS and TBI is shown in Figure 2. Determining the molecular regulators that lead to persistent ER stress activation will open the door for novel modulation of ER stress at extended time points.

**ER STRESS RESPONSE IN MODELS OF ISCHEMIC STROKE**

Protein aggregation can result when transient cerebral ischemia disrupts blood supply and interrupts energy metabolism [19]. Oxygen deprivation causes the ER to swell due to the accumulation of aberrant proteins [51]. The physical
effects of ischemia trigger the UPR and inhibit protein synthesis in those affected areas [52,53]. The UPR has been implicated as an important player during both transient and focal ischemia and is preceded by an intracellular Ca\(^{2+}\) increase [54]. Usually the ER uses Ca\(^{2+}\) pumps to reduce high Ca\(^{2+}\) levels within the cell, but sometimes the Ca\(^{2+}\) load becomes too high for the ER to handle and the overloaded Ca\(^{2+}\) pumps become damaged [55]. Tissues lacking the necessary oxygen from cerebral blood supply are subject to a disruption in Ca\(^{2+}\) homeostasis due to loss of function in the energy-dependent ER Ca\(^{2+}\) efflux pump [56]. Global IS initially triggers a robust UPR accompanied by increases in the pro-survival protein BiP, while more focal injury triggers apoptosis [57]. A model of middle cerebral artery occlusion, another model of IS, exhibited increased expression of the active form of caspase-12 [58], indicating an ER-dependent apoptotic response. A third model of IS, bilateral carotid artery occlusion, showed increased expression of CHOP and BiP with a longer transition to apoptosis [38]. Each model shows that stroke upregulates markers of the UPR and subsequent apoptosis, but the transition to apoptotic pathways are dependent on duration or severity of injury. Ongoing therapeutic investigation should focus on energy maintenance, inflammation attenuation, and controlling apoptosis.

**ER STRESS RESPONSE IN MODELS OF TRAUMATIC BRAIN INJURY**

Stroke and TBI have several shared mechanisms of injury progression. After TBI, inflammatory responses are triggered [59], apoptotic cascades commence [60], and the UPR is induced [5]. Despite these shared mechanisms, there are subtle differences that differentiate both the primary and secondary injuries. TBI triggers rapid changes in glutamate signaling and oxidative stress with long-term sequela mediated through nuclear factor regulators. The consequence of injury progression has recently been associated with long-term behavioral and pathologic changes within the CNS. Mechanistic progression is also highly dependent upon injury severity. TBI presentation is variable with severe injuries causing loss of consciousness while subconcussive injuries produce limited acute symptoms. The brain’s response to TBI is more globally distributed than stroke with a vast number of perivascular foci. In contrast to the penumbra seen in stroke, pathologic changes are scattered throughout the brain and highly dependent on axonal shearing and gliosis. The molecular mechanisms of TBI are being pursued by a large number of researchers due to the increased awareness of how these changes contribute to chronic neuropsychiatric symptoms [16,61].

TBI produces an excess of ROS [62] and can accumulate damaging levels of intracellular Ca\(^{2+}\) [63]. Over time TBI can also cause tau hyperphosphorylation through mechanisms that are not completely understood [16]. Although these factors are common among all neural injury models, in TBI models, these factors are activated immediately following injuries and have the potential to advance profoundly over time. The cellular repair mechanisms are more drawn out, and may contribute to neurodegenerative disease [21,64]. How these effects manifest into chronic disease states is an area of ongoing investigation.

The most common cellular response mechanism to be studied following TBI is inflammation, and more specifically how inflammation leads to subsequent apoptosis [65]. The UPR has only recently been addressed by TBI investigators because

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**Figure 2** Role of UPR Transcription Factors following Neural Injury. Four transcription factors are activated following neural injury: ATF4, XBP1, ATF6 and NF-kB. ATF4 up regulates proteins involved in reducing the proteins in the ER. XBP1 upregulates proteins involved in degrading misfolded proteins. ATF6 up regulates proteins involved in refolding proteins within the ER. NF-kB up regulates proteins that facilitate protein production.
of its new found link to neurodegeneration [66]. The brains of professional athletes and military veterans are currently being examined by new imaging modalities to discover underlying causes behind neuropsychiatric symptoms such as post-traumatic stress disorder, chronic traumatic encephalopathy, and Alzheimer’s disease (AD). The timing and duration of the UPR and how exactly it develops into a neurodegenerative phenotype warrants further investigation.

ER STRESS AND NEUROINFLAMMATION

Neuroinflammation is another downstream cascade resulting from the inability of the ER to remove intracellular Ca\(^{2+}\) accumulation following neural injury [67]. Both IS and TBI cause an acute upregulation of inflammatory processes; in particular, the NF-kB inflammation pathway has been shown to be activated after neural injury [68,69]. NF-kB contributes to the release of anti-inflammatory cytokines. These cytokines attract microglia and astrocytes to the site of injury in order to scavenge dead or severely damaged neurons [70]. However, when NF-kB has been activated over a long period of time, pro-inflammatory cytokines are released causing detrimental effects—this pathway has recently been linked to ER stress [71]. The following sub-sections review how neuroinflammation is manifested during IS and TBI.

Stroke

After an IS event, NF-kB mediates many genes associated with inflammation [68,72]. The UPR, in part, regulates NF-kB and subsequent inflammation [73]. Attempts have been made to administer anti-oxidants as a therapy to mitigate NF-kB induction of pro-inflammatory cytokines following IS [70]; however, this approach has failed to translate to the clinic. In fact, a direct link between ER stress and stroke-induced inflammation has yet to be determined. Because the inflammatory response and UPR both act in a biphasic manner following ischemic insult [41], additional emphasis should be placed on combination therapy targeting both mechanisms, during the same phase of activation.

TBI

Inflammation is also an important component of TBI exposure [74,75]. UPR mechanisms are triggered following TBI and have been linked to a concomitant inflammatory response. For instance, the UPR is shown to phosphorylate eIF2α following controlled cortical impact [6]. Consequently, activated eIF2α suppresses the translation of endogenous inhibitors of nuclear factor kappa B (IKK) [76], and thereby indirectly increases the levels of NF-κB. Therefore, it appears that the UPR can augment NF-κB activation and promote cell survival in the short term, but the role of NF-κB during prolonged ER stress has not been elucidated. Inflammation and ER stress are closely tied through the NF-κB pathway and future work will examine these intricate interconnections.

As mentioned previously, NF-κB plays an important role in mediating inflammatory cascades following TBI. A prominent study showed that overexpression of Tumor Necrosis Factor Receptor-Associated Factor 6 (TRAF6) led to activation of both NF-κB and its’ upstream component Akt [77]. Interestingly, Akt phosphorylation was attenuated by overexpression of the ER stress marker, GADD34, in a rodent TBI model [5]. In the same study by Farook et al., GADD34 was proposed to sequester TRAF6 and promote Akt degradation; thereby exacerbating neuronal death [5]. A summary of NF-kB activity following neural injury has been simplified in Figure 3. Such a paradox opens the possibility for counter-regulation of neuroinflammation by targeting arms of the UPR. As such, a connection between NF-kB activation and ER stress following TBI is likely and warrants future investigation.

PHARMACOLOGICAL TOOLS USED TO RESEARCH ER STRESS FOLLOWING NEURAL INJURY

Stroke

The overarching goal in stroke research is to prevent as much secondary injury as possible after an ischemic event. Two strategies have been employed: preserve the penumbra surrounding the necrotic core [78] and equally important extend the treatment window for thrombolytics [79]. More clinically-relevant models of stroke are now being used to simulate an IS as a physician would experience from a human patient. Researchers insert blood clots into the brain vasculature (mimicking an embolism), treat with thrombolytics (tPA), and assess post-operative recovery (mNSS) [80]. Even with the success of preclinical studies, the treatments that work in rodents have not translated well to clinical human patients. New treatment options for IS are being evaluated that target the UPR and inflammation (Table 1). Combination treatments that address multiple sites on these complicated pathways will likely have better effects than single-target acute acting drugs. Once a stronger connection between ER stress and inflammation has been established, ideal targets can be mapped for regions of shared overlap.

TBI

The primary effects of TBI are acute cell death from glutamate excitotoxicity. Targeting glutamate-mediated toxicity is limited due to the short onset of initiation. The secondary effects of injury should therefore be the foremost target of therapeutic intervention. Immediate secondary effects include: axonal sheering [15], blood brain barrier disruption [81] and intracellular Ca\(^{2+}\) accumulation [55]. These effects are common following TBI but can potentially be attenuated with timely pharmaceutical intervention. Prolonged secondary events can persist and include: UPR activation [5], apoptosis [6,52] and inflammation [83]. In the past decade, experimental pharmacologic interventions targeting these secondary events have become prominent. Although these events are naturally associated with TBI progression, they have a higher potential to be attenuated if the right therapies are implemented, at the right time. We provide a list of the most frequently used ER stress modulators for TBI research in Table 2. With the recent linkage between UPR activation and subsequent neurodegeneration [8], the focus of research pharmacotherapy has transitioned towards mitigation of chronic neural decline. While tau toxicity itself is debated, tau is a well-known marker concurrently expressed during neurodegeneration. The ultimate goal is to diminish neurodegeneration by treating secondary TBI pathways early.

Docosahexaenoic acid (DHA), an important component of omega-3 fatty acids, has recently garnered attention as an ER stress inhibitor. DHA has been reported to block inositol triphosphate receptor in the ER and therefore prevent intracellular Ca\(^{2+}\) accumulation and subsequent UPR activation during IS [59]. A
Central Huber et al. (2014)

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Figure 3 The UPR leads to NFκB activation following Neural Injury. Each adaptive arm of the UPR can lead to translocation of the p50/p65 complex (NF-κB) following intracellular Ca\(^{2+}\) accumulation. ROS accumulation also leads to NF-κB nuclear translocation. TRAF6 overexpression phosphorylates Akt and activates NF-κB. GADD34 (Upregulated via the PERK arm of the UPR) sequesters TRAF6 to inhibit Akt phosphorylation and subsequent NF-κB activation.

Table 1: Pharmacological tools used to research ER stress in IS models.

<table>
<thead>
<tr>
<th>Pharmacological Tools</th>
<th>Mechanism of Action</th>
</tr>
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<tbody>
<tr>
<td>Edaravone</td>
<td>ROS scavenger; Inhibits P-eIF2α, CHOP and caspase-12 (101)</td>
</tr>
<tr>
<td>Dantrolene</td>
<td>Ryanodine receptor antagonist; reduces Ca(^{2+}) &amp; apoptosis (102)</td>
</tr>
<tr>
<td>Sodium Phenylbutyrate</td>
<td>Histone Deacetylase inhibitor; attenuates ER stress (103)</td>
</tr>
<tr>
<td>Parecoxib</td>
<td>Cyclooxygenase-2 inhibitor; upregulates BiP(104)</td>
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Abbreviations: IS: Ischemic Stroke; ER: Endoplasmic Reticulum; ROS: Reactive Oxygen Species; BiP: Binding Immunoglobulin Protein; eIF2α: Eukaryotic Initiation Factor 2 Alpha; CHOP: C/E Homology Protein

Table 2: Pharmacological tools used to research ER stress in TBI models.

<table>
<thead>
<tr>
<th>Pharmacological Tools</th>
<th>Mechanism of Action</th>
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<tbody>
<tr>
<td>Salubrinal</td>
<td>Inhibits eIF2α dephosphorylation; inhibits PPI(105)</td>
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<tr>
<td>Guanabenz</td>
<td>Inhibits PPI complex formation; GADD34 sequestration (106)</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>Known inhibitor of ROS (107); Phase II trials for AD (108)</td>
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<tr>
<td>Phenazine</td>
<td>Unknown; diminishes neurodegenerative phenotype (109)</td>
</tr>
<tr>
<td>Docosahexaenoic Acid</td>
<td>Unknown; attenuates ER stress and tau phosphorylation(6)</td>
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</tbody>
</table>

Abbreviations: TBI: Traumatic Brain Injury; ER: Endoplasmic Reticulum; ROS: Reactive Oxygen Species; BiP: Binding Immunoglobulin Protein; eIF2α: Eukaryotic Initiation Factor 2 Alpha; GADD34: Growth Arrest and Damage Inducible Agent 34; PPI: Protein Phosphatase 1; AD: Alzheimer’s disease

follow up study was conducted by Begum and colleagues (2014), in which they show that DHA attenuates the UPR following TBI and also mitigates tau protein phosphorylation. Pre-clinical models are important in order to understand mechanism of intervention, but clinical trials will be necessary to capitalize dosing and timing strategies.

**ER-MEDIATED APOPTOSIS FOLLOWING PROLONGED ER STRESS ACTIVATION**

When unfolded proteins are not properly refolded or degraded, stress response genes are upregulated. These stress response genes activate proteins that proteolytically cleave caspases and therefore lead to programmed cell-death [7]. Caspase-12 is specific to the endoplasmic reticulum and triggers apoptosis through downstream activation of caspase-3[84]. Prolonged ER stress inevitably leads to caspase-12 activation and apoptosis in models of IS [85]. Models of TBI have also shown upregulation of Caspase-12 mRNA expression [86], indicative of UPR-mediated apoptosis. This UPR-mediated caspase activity may also be involved in the pathogenesis of AD [87]. Apoptosis
is not purely detrimental to the damaged brain considering the heightened energy demands following IS or TBI. By limiting energy expended on severely damaged cells, the brain can preserve function to surviving cells. Apoptosis can however induce immunonecxtotoxicity leading to glutamate release into the surrounding milieu [88]. Moreover, immunonecxtotoxicity has been implicated in tau hyperphosphorylation and neurodegeneration [89]. Therefore, the long-term effects of apoptosis must be weighed against the benefits of short-term energy conservation when considering therapeutic intervention.

The UPR is triggered following injury in order to maintain cellular homeostasis [66]. The UPR shuts down protein folding, but if left unchecked these unfolded proteins can have long-term detrimental effects on the cell. Through interaction with BiP and the subsequent activation of the three arms of the UPR, unfolded proteins push CHOP above threshold [90]. Importantly, CHOP can cleave and activate the pro-apoptotic protein, caspase-12, on the ER membrane [91]. Caspase-12 interacts with apoptosis signal-regulating kinase 1 (ASK1) to facilitate the cleavage of caspase-3 [92]. In addition CHOP decreases the anti-apoptotic Bcl-2 within the mitochondria while facilitating the release of ROS [93]. The UPR can also interact with the mitochondria to initiate apoptosis through cytochrome C dissociation [94]. Even inflammatory component NF-kB has been implicated in Bcl-2 activation [95], further supporting the notion of a link between the UPR, inflammation and apoptosis. While important in mediating acute injury cascades, the UPR is also heavily involved in neurodegenerative disease.

**ER STRESS AND NEURODEGENERATION AFTER NEURAL INJURY**

ER stress has recently been linked to chronic diseases such as AD, Parkinson’s disease, IS, and prion disorders [96]. In AD patients, ER stress enhances neuronal autophagy and tau hyperphosphorylation [64]. However, any causal link between ER stress and chronic neurodegeneration following neural injury remains poorly understood. The triggering event for persistent UPR is metabolic dysfunction that leads to aberrant protein accumulation [97]. Parkinson’s disease results in UPR-mediated dopaminergic cell apoptosis [98]. After an IS event, the UPR is initially protective but can be detrimental over along period of time [54]. The second arm of the UPR prevents vascular regeneration, and also triggers apoptosis if ER stress persists [59,99]. Another degenerative disease known as Prion disease is unique in the fact that the disease accumulates aberrant proteins within the ER lumen from synaptic transportation deficiencies[100-109].

A group has successfully shown UPR activation in prion diseased rodents providing yet another link between ER stress and chronic neurodegeneration [8]. Although much is yet to be learned regarding ER stress and neurodegenerative disease, the therapeutics targeting the UPR may help mitigate a cells progression towards neurodegeneration.

**CONCLUSION**

In summary, the UPR is activated following both IS and TBI. The same stress pathways are activated, the same inflammatory processes are triggered, and the same apoptotic results ensue. Both injury models produce excess ROS, increased intracellular Ca²⁺ levels, and induce inflammation; however, the timing, severity, and duration all vary from model to model. It is difficult to prevent the immediate effects of IS or TBI, but we can attempt to mitigate the secondary effects that follow. Preventative therapies, mostly pharmacological agents, have been employed in pre-clinical models of neural injury, but nothing has yet to successfully translate to the clinic. The approach has been to mediate apoptosis and reduce neurodegeneration. However, the underlying link between ER stress, apoptosis and neurodegeneration is not yet fully understood. Research is now being focused on the most effective treatment strategies to suppress chronic effects resulting from neural injury. Of particular importance is transferring preclinical success to effective treatment options for human care. Evidence suggests that combining therapeutic options that target multiple injury components at the ideal time window will be the most effective strategy going forward.

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