

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Glial Cells, Inflammation and Heat Shock Proteins in Cerebral Ischemia

Vivianne L. Tawfik, Robin E. White and Rona Giffard

*Department of Anesthesia, Stanford University School of Medicine, Stanford, CA  
USA*

## 1. Introduction

Each year approximately 795,000 people in the United States suffer a new or recurrent stroke and it is the third leading cause of death after heart disease and cancer (Lloyd-Jones et al., 2010). The estimated cost of stroke was \$73.7 billion nationwide in 2010, the majority of which was related to payments for inpatient care and rehabilitation for significant morbidity (hemiparesis, aphasia and loss of independence). Stroke is broadly divided into two categories: ischemic and hemorrhagic. The former is related to too little blood supplied to the brain, secondary to thrombus or embolus, and the latter results from excess blood escaping into the cranial cavity. Ischemic brain injury represents conditions including focal ischemia, with subsequent loss of blood flow and nutrients to one area of the brain, and global ischemia, as seen in cardiopulmonary arrest and resuscitation which, when brief, results specifically in neuronal death in the CA1 region of the hippocampus (Pulsinelli, 1985). In either case, decreased cerebral blood flow initiates a cascade of ATP depletion, ion gradient disruption, excessive glutamate release, formation of reactive oxygen species and increased lactic acidosis that leads to neuronal death (Doyle et al., 2008). To date, the only FDA-approved treatment for focal ischemic stroke is recombinant tissue plasminogen activator which aims to restore blood flow by recanalization of the occluded vessel (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). In global ischemia, multiple clinical trials have demonstrated that therapeutic hypothermia increases survival and improves neurologic outcome (Bernard et al., 2002; Sahota and Savitz, 2011; The Hypothermia after Cardiac Arrest Study Group, 2002). Though the exact mechanisms remain unclear, effects on several different pathways have been observed. In spite of this one treatment modality, survival to hospital discharge after cardiac arrest and attempted resuscitation remains a dismal 5-18% depending on the cause and rapidity of the response (de Vreede-Swagemakers et al., 1997; Eckstein et al., 2005).

To date, more than one hundred potential pharmacological strategies for stroke have failed to show improved outcome in phase III trials. As such, the role of central nervous system glial cells has recently come under scrutiny as work focused on neurons alone has failed to reverse neuronal death in ischemic areas of the brain. Glial cells (microglia, astrocytes and oligodendrocytes) constitute over 70% of the total cell population in the CNS and are active contributors to neuromodulatory, neurotrophic and neuroimmune events in the brain and

spinal cord (Pellerin, 2005; Reichenbach and Wolburg, 2005). Once thought of merely as neuronal support cells, astrocytes and microglia, in their physiologic role, dynamically control synaptic function and neuronal activity by performing a variety of crucial functions. Microglia, the intrinsic macrophages of the CNS, provide immune surveillance against invading pathogens or nervous system insults (Aloisi, 2001) while astrocytes regulate synaptic glutamate levels, contribute to the blood-brain-barrier and supply neuronal growth factors (Liberto et al., 2004). Given their complex involvement in normal CNS function, glial cells must be considered in any strategy focused on neuronal preservation after ischemic injury.

Heat shock proteins (HSP) are a phylogenetically conserved group of chaperones that assist in ATP-dependent protein folding, translocation across membranes, suppression of protein aggregation, presentation of substrates for degradation and modulate a host of other intracellular processes (Hartl, 1996). The 70 kDa heat shock protein family (HSP70) is the most extensively studied group of chaperones and consists of at least twelve constitutive and inducible proteins which aid in a coordinated response to cellular stressors. The most important members include: the constitutively expressed primarily cytosolic Hsc70/Hsp73, the heat inducible cytosolic form Hsp70/72, the glucose regulated mitochondrial protein Grp75/mortalin/mtHsp70 and the endoplasmic reticulum glucose regulated protein Grp78/BiP. The HSP70s are structurally comprised of a 44 kDa amino-terminal ATPase domain, 18 kDa carboxyl-terminal substrate-binding domain and a more variable 10 kDa segment that terminates in the highly conserved EEVD sequence that regulates intramolecular interactions and ATPase activity (Freeman et al., 1995). Our laboratory has demonstrated that the carboxyl-terminal domain of Hsp72 is sufficient to protect astrocytes from oxygen-glucose deprivation and decrease infarct volume after transient middle cerebral artery occlusion (MCAO) (Sun et al., 2006b).

Extensive work using overexpression and knockout of HSP70 family members has highlighted integral cytoprotective, anti-apoptotic and immune regulatory roles for these proteins. Induction of Hsp70/72 by heat stress or targeted overexpression in multiple experimental disease models including stroke (Rajdev et al., 2000), sepsis (Ryan et al., 1992), renal injury (Jo et al., 2006) and acute lung injury (Villar et al., 1994) demonstrated decreased organ injury and enhanced survival. Lee et al. (2001) showed that while *hsp70.1* knockout mice have a normal baseline phenotype; cerebral infarct volume was 30% larger and mortality was higher than in wild type littermates after focal ischemia. In addition, using a combined *hsp70.1/3* knockout (the two genes are separated by only 8kb on chromosome 17 and show 99% homology), Lee et al. (2004) later determined that cytochrome *c* release into the cytosol and levels of activated caspase-3 were increased after MCAO suggesting that Hsp 70/72 plays a role in preventing initiation of apoptosis after injury. The *hsp70.1/3* knockout also exhibited an enhanced inflammatory response to cecal perforation and ligation (an animal model of acute respiratory distress syndrome/sepsis) as evidenced by increased NF- $\kappa$ B activation, TNF- $\alpha$  and IL-6 expression and lung injury highlighting a role in controlling immune function in injury states (Singleton and Wischmeyer, 2006). In this same model, Weiss et al. (2002) demonstrated that targeted overexpression of Hsp70 in rat lung significantly attenuated interstitial and alveolar edema, protein exudation and dramatically decreased neutrophil accumulation leading to improvement of acute respiratory distress syndrome.

Overall, this chapter seeks to review the importance of astrocytes and microglia in the post-ischemic inflammatory response and the role of heat shock proteins in modulating inflammation and outcome after cerebral ischemia.

## 2. Microglia: Immune cells of the CNS

Microglia are monocyte-derived CNS tissue macrophages that are phenotypically adapted to the neural environment. As such, they are characterized by minimal phagocytic activity and low expression of the macrophage-specific antigen, CD45 (Kreutzberg, 1996) which may be used to differentiate resident microglia from infiltrating macrophages (Babcock et al., 2003). Microglia constitutively and inducibly express a variety of immune-related receptors on their surface including cytokine, chemokine, prostaglandin, pattern recognition and complement receptors (Aloisi, 2001). In addition, microglia can function as antigen presenting cells, and through CD40 on their membranes can form an “immunological synapse” with CD40L-expressing T-cells recruited centrally (Gerritse et al., 1996). As a result, microglia can respond to diverse stresses by performing innate immune functions such as phagocytosis, and can release potentially beneficial factors such as glial-derived neurotrophic factor (GDNF).

Microglia can release a host of factors to protect the CNS; however, when activated after ischemia by necrotic cell debris and other substances, they can produce free radicals, proinflammatory cytokines (IL-1 $\beta$ , TNF $\alpha$ , IL-6 and interferon- $\gamma$ ), reactive oxygen species, matrix metalloproteinases and glutamate in an aberrant fashion (Lucin and Wyss-Coray, 2009; Yenari et al., 2010). Such compounds are instrumental in the subsequent activation of astrocytes (see below), induction of cell adhesion molecules and T-lymphocyte recruitment into the CNS following various injuries (Liu and Hong, 2003; Sweitzer et al., 2002). Several studies have been performed testing inhibition of microglial activation as a strategy to protect neurons following ischemic injury. Transgenic mice lacking the pattern recognition receptor TLR4 (Hyakkoku et al., 2010), mice overexpressing the anti-inflammatory cytokine IL-10 (De Bilbao et al., 2009) and treatment with a neutralizing antibody against TNF- $\alpha$  (Barone et al., 1997), all resulted in suppressed microglial activation and significantly decreased infarct size following focal cerebral ischemia. Taken together, these studies suggest that inhibition of the post-ischemia inflammatory response is a viable option for stroke treatment. Although microglia quickly respond to ischemia by producing proinflammatory cytokines (Yenari et al., 2010), proliferating (Denes et al., 2007) and exhibiting an altered cell morphology (Tanaka et al., 2003), only a small percentage (1-8%) of the microglia in the corpus callosum and lesion penumbra, and no microglia in the lesion core, express Hsp72 early after focal ischemia (Soriano et al., 1994). This indicates that a robust increase in Hsp72 protein expression is not a normal aspect of the post-ischemic microglial phenotype.

Interestingly, the majority of inflammatory mediators produced by microglia after stroke are produced by NF $\kappa$ B pathway activation (Yenari et al., 2010). Hsp72 has been implicated in modulation of inflammation by suppressing NF $\kappa$ B through multiple interactions. Previous work has shown that Hsp72 directly binds to the NF $\kappa$ B:I $\kappa$ B complex, thus preventing I $\kappa$ B phosphorylation and subsequent NF $\kappa$ B activation (Feinstein et al., 1996; Zheng et al., 2008). Ran et al. (2004) further demonstrated that Hsp72 binds directly to I $\kappa$ B kinase- $\gamma$  (IKK- $\gamma$ ), an essential regulatory component of the IKK complex, blocking activation of I $\kappa$ B and release of

NFκB. Mice overexpressing Hsp72 have decreased infarcts compared to their wild-type counterparts, along with attenuated microglial activation and TNF-α production (Rajdev et al., 2000; Zheng et al., 2008). Microglia isolated from these mice showed decreased toxicity towards cultured astrocytes, accompanied by decreased NFκB signaling. Thus, the use of heat shock proteins to inhibit NFκB signaling in microglia may be an effective treatment for stroke by inhibiting a plethora of downstream factors that ultimately lead to further glial cell activation and neuronal cell death.

### 3. Astrocytes: Multiple roles in physiology & pathophysiology

Historically, astrocytes were considered to be passive elements in the CNS and neurotransmitter receptor expression was believed to be solely a characteristic of neurons. To the contrary, astrocytes express a variety of receptors on their surface, including metabotropic glutamate receptors, GABA receptors, adenosine receptors and the mu, delta and kappa opioid receptors, among others (Kettenmann and Steinhauser, 2005). In addition, these cells express a variety of ion channels on their surface, including Ca<sup>2+</sup> channels. These are important because astrocytes are thought to function as part of a syncytia linked by connexins in order to transfer information in the form of ATP and Ca<sup>2+</sup> (Kielian, 2008). In a model of ischemia, Cotrina et al. (1998) demonstrated that gap junctions remain open after oxygen-glucose deprivation (OGD) and may contribute to infarct evolution through direct astrocytic intercellular communication.

Astrocytes derive from the neuroectoderm and express a series of “marker antigens” during development such as the cytoskeletal protein vimentin and nestin (Eliasson et al., 1999) and the fatty acid binding protein brain lipid binding protein (Schmid et al., 2006). Once they reach their adult phenotype, other proteins are expressed including Aldh1L1 and glial fibrillary acidic protein (GFAP) (Cahoy et al., 2008). This intermediate filament is commonly considered to be astrocyte-specific, though it may also be found on reactive choroid plexus epithelium cells and neuronal precursor cells (Reichenbach and Wolburg, 2005) and there are also astrocyte populations that are GFAP negative (Kimelberg, 2004). GFAP functions as a structural protein and enhancement of GFAP remains the mainstay for demonstrating astrocytic reactivity in the CNS (Eng et al., 2000), however, it is important to note that only 15% of the total astrocyte cell volume is labeled with GFAP (Bushong et al., 2002). Remarkably, GFAP knockout mice do not exhibit an altered phenotype at baseline; however, after trauma, astrocyte hypertrophy is suppressed, scar formation is less organized and healing is slowed (Pekny et al., 1995; Pekny and Pekna, 2004). In further work using a lesion model of the entorhinal cortex, Wilhelmsson et al. (2004) demonstrated that double GFAP<sup>-/-</sup> Vim<sup>-/-</sup> mice display increased neuronal loss in the dentate gyrus at day 4 post-injury but enhanced synaptic regeneration at day 10. These data suggest a two-part response of reactive astrocytes to CNS injury: beneficial for neuronal survival in the initial post-injury period and detrimental to CNS regeneration in the recovery phase (Pekny and Nilsson, 2005).

#### 3.1 Role in the blood-brain barrier

Astrocytes play an integral role in the structure of the blood-brain barrier (BBB), which limits the entry of circulating elements into the nervous system. Though recent data suggests that pericytes, not astrocytes, are required for the formation of the BBB during



development (Daneman et al., 2010), ablation of astrocytes in the process of CNS restoration leads to failure of blood-brain barrier repair, an enhanced infiltration of leukocytes and subsequent excitotoxic neuronal death (Bush et al., 1999). In addition, astrocytes have been postulated to mediate functional hyperemia, the coupling of neuronal activity with increased cerebral blood flow, via changes in intracellular calcium in astrocytic endfeet leading to release of vasoactive substances (cyclooxygenase, adenosine) and modulation of adjacent arterioles (Iadecola and Nedergaard, 2007; Takano et al., 2006). It is estimated that 56% of rat cortical synapses are ensheathed by astrocyte domains (Chao et al., 2002) and an individual astrocyte occupies an exclusive, non-overlapping territory; each interfacing with the vasculature and thousands of synapses suggesting a complex process of synaptic integration (Bushong et al., 2004). Indeed, mice lacking GFAP exhibit increased infarct following ischemia, possibly due to blood-brain barrier and cerebral blood flow dysfunction (Nawashiro et al., 2000). The implication is that following ischemia, astrocytes are poised to influence penumbral blood flow and provision of neuronal nutrients in a coordinated fashion.

### 3.2 Astrocytes and ischemic injury

While it was established over a hundred years ago that neurons of the CA1 region of the hippocampus are selectively vulnerable to forebrain ischemia (Pulsinelli, 1985); evidence for injury to astrocytes has been more recent (Petito et al., 1998). The two main types of astrocytes found in the CNS are protoplasmic astrocytes found in the gray matter and fibrous astrocytes found in the white matter. This is important because astrocytes isolated from different brain regions exhibit varying sensitivity to oxygen-glucose deprivation (OGD) with striatal cells most vulnerable followed by hippocampal and cortical astrocytes (Xu et al., 2001). *In vivo*, using the middle cerebral artery occlusion (MCAO) model of focal ischemia, Lukaszevicz et al. (2002) demonstrated selective degeneration of protoplasmic cortical astrocytes with associated breakdown of the blood brain barrier. Yu et al. (1989) first demonstrated that cultured astrocytes are sensitive to hypoxia, exhibiting swelling and 80% suppression of glutamate uptake after 12-24 hours of oxygen deprivation. Using *in situ* hybridization and immunohistochemistry, Liu et al. (1999) demonstrated an early decline in mRNA and protein for GFAP in the ischemic core after middle cerebral artery occlusion (MCAO) with a corresponding increase in astrocyte markers in the penumbra, both of which temporally preceded neuronal death. In agreement, Zhao et al. (2003) showed early loss of GFAP after traumatic brain injury. Early *in vitro* work determined that co-culture of neurons with astrocytes protected them from OGD; specifically, when cultured alone and exposed to 4 hour of OGD only 5% of neurons survived compared to 75% survival in mixed cultures (Vibulsreth et al., 1987). Neurons co-cultured with astrocytes have also been shown to survive exposure to 100-fold higher concentrations of glutamate (Rosenberg and Aizenman, 1989). Taken together, these studies suggest that therapeutics aimed at maintaining astrocyte viability and function may protect neurons from ischemic injury.

### 3.3 Astrocytes as regulators of synaptic glutamate

Another key function of astrocytes is the control of extracellular glutamate homeostasis through sodium-dependent uptake via the excitatory amino acid transporters (EAATs) (Danbolt, 2001). The glutamate-aspartate transporter (GLAST/EAAT1) and the glutamate transporter-1 (GLT-1/EAAT2) are primarily localized in astrocytes. GLT-1 is the most

studied astrocyte transporter and is suggested to be responsible for over 90% of synaptic glutamate clearance (Tanaka et al., 1997). Dysregulation of synaptic glutamate clearance by these transporters has been implicated in many disease processes (Gegelashvili and Schousboe, 1997; Maragakis and Rothstein, 2001; Rothstein et al., 1996). For example, glutamate levels have been shown to increase 50 times from baseline after ischemia and glutamate efflux from astrocytes has been suggested to occur by reversal of glutamate transport (Mitani et al., 1994; Seki et al., 1999). Transient MCAO leads to downregulation of GLT-1 which precedes neuronal death and antisense knockdown of GLT-1 exacerbates neuronal death in the same model (Rao et al., 2001a; Rao et al., 2001b; Rao et al., 2000). Furthermore, using pre-treatment with ceftriaxone, a known inducer of GLT-1, Chu et al. (2007) demonstrated a dose-dependent decrease in infarct volume and levels of the proinflammatory cytokine TNF $\alpha$  after MCAO. In addition, work from our laboratory showed that upregulation of GLT-1 in astrocytes using ceftriaxone decreases CA1 neuronal cell death in a global ischemia model (Ouyang et al., 2007). Complete knock-out of GLT-1 results in spontaneous seizures, selective death of CA1 neurons and 20% survival of animals at 12 weeks (Tanaka et al., 1997) and mice lacking GLT-1 display enhanced neuronal death after brief ischemia compared to wild type controls (Mitani and Tanaka, 2003). These findings underline the importance of exquisite regulation of synaptic glutamate by astrocytes in maintaining neuronal integrity.

## **4. Heat shock proteins affect astrocyte regulation of ischemia**

### **4.1 A role for Hsp72 in ischemia**

Our laboratory has been particularly interested in the role of astrocytic heat shock proteins as regulators of ischemic injury. Initial studies demonstrated induction of Hsp72 in cultured astrocytes exposed to heat shock or OGD (Bergeron et al., 1996) and further work confirmed that Hsp72 overexpression in astrocytes exposed to glucose deprivation (Xu and Giffard, 1997) or oxygen-glucose deprivation (Papadopoulos et al., 1996) was cytoprotective. Interestingly, overexpression of Hsp72 in astrocytes was shown to protect co-cultured neurons from ischemic injury (Xu et al., 1999); highlighting the integral role of astrocytes in neuronal homeostasis and survival. As discussed above, we have also demonstrated that the carboxyl-terminal domain of Hsp72 is sufficient to protect astrocytes from oxygen-glucose deprivation by suppressing protein aggregation and further decreases infarct volume after transient middle cerebral artery occlusion (MCAO) (Sun et al., 2006b). Astrocytes in the CA1 region of the brain, which is particularly sensitive to forebrain ischemia, lose glutamate transporter expression and activity prior to the death of CA1 neurons (Chen et al., 2005; Ouyang et al., 2007; Yeh et al., 2005). We have shown that astrocyte-targeted overexpression of Hsp72 not only protects CA1 neurons from transient forebrain ischemia, but also preserves GLT-1 immunoreactivity in the region (Xu et al., 2010) suggesting a possible mechanism for the observed protection.

### **4.2 Hsp72 as a regulator of apoptosis**

Multiple studies have highlighted a neuroprotective role of Hsp72 overexpression in models of ischemia (Hoehn et al., 2001; Rajdev et al., 2000; van der Weerd et al., 2005). The mechanism was initially attributed to the known chaperone functions of Hsp72 including maintaining correct protein folding and inhibiting aggregation, however, a body of work

has now emerged that indicates a direct role for Hsp72 in regulation of cell death by apoptosis and potentially even necrosis (Giffard and Yenari, 2004). Mitochondria are central to both cell death pathways; severe ischemia renders mitochondria unable to produce ATP and in less extreme stress conditions, mitochondria may increase production of reactive oxygen species (ROS), lose membrane potential and undergo changes in respiratory function (Dugan and Kim-Han, 2004). Ischemia can activate mitochondrial cytochrome *c* which translocates to the cytosol where it interacts with Apaf1 to form the apoptosome and activate caspase 9, initiating a cascade leading to DNA fragmentation (Chan, 2004; Leist and Jaattela, 2001). We have shown that overexpression of Hsp72 in cultured astrocytes subjected to glucose deprivation leads to decreased formation of ROS, stabilization of the mitochondrial membrane potential and prevention of increases in state IV respiration suggesting decreased cytochrome *c* release and activation of apoptosis (Ouyang et al., 2006). Furthermore, in the MCAO model of ischemia, we have shown that transfection of Hsp72 leads to inhibition of apoptosis-inducing factor (AIF) translocation to the nucleus thereby blocking caspase-independent apoptosis (Sun et al., 2006b). This is supported by previous work by Ravagnan et al. (2001) demonstrating that Hsp72 protects Apaf<sup>-/-</sup> cells against death via an interaction with AIF. For a comprehensive review of the role of Hsp72 in cell death please see Giffard et al. (2008).

#### 4.3 Mitochondrial protection and mortalin/mitochondrial Hsp70

Mitochondrial dysfunction leading to a loss of ATP production impairs many of the energy-demanding neuroprotective functions of astrocytes after ischemic injury including ion homeostasis and neurotransmitter turnover (Bambrick et al., 2004). Mortalin forms part of the mitochondrial protein import machinery by binding a translocase in the inner membrane to form an ATP-dependent motor (Voos et al., 1999) and while it is not heat inducible it has been shown to increase after a variety of other stressors including glucose deprivation, oxidative stress and focal cerebral ischemia (Hadari et al., 1997; Lee, 2001; Massa et al., 1995).

Using LXS<sup>N</sup>-mortalin-transduced astrocytes, our laboratory has shown that overexpression of mortalin produces mitochondrial protection after glucose deprivation (Voloboueva et al., 2008). Specifically, we found decreased hydroethidine fluorescence (an indicator of the accumulation of reactive oxygen species (ROS)) and preserved mitochondrial membrane potential as measured by tetramethyl rhodamine staining (TMRE), a dye whose sequestration by mitochondria depends on the mitochondrial membrane potential, in astrocytes expressing increased levels of mortalin. In addition, mortalin overexpression preserved ATP levels in astrocytes subjected to oxygen-glucose deprivation and enhanced cell survival. In a more clinically relevant model of stroke, middle cerebral artery occlusion (MCAO), we further investigated the role of mortalin in mitochondrial protection. Rats overexpressing mortalin in astrocytes and neurons by direct intraventricular injection of a DNA plasmid encoding mortalin were subjected to MCAO and found to have a reduction in infarct area, decreased ROS and lipid oxidation compared to vector-transfected controls. Similar to our *in vitro* data we showed that mortalin overexpression reduced the ischemia-induced depletion of ATP and maintained electron transport chain complex IV activity (Xu et al., 2009).

To investigate the specific role of astrocytic mitochondrial inhibition in ischemia we treated astrocyte cultures with the Krebs cycle inhibitor, fluorocitrate (Voloboueva et al., 2007).



After glucose deprivation, astrocytes treated with fluorocitrate showed depletion of ATP, cell death and suppressed glutamate uptake within 3 hours. In addition, we demonstrated that inhibition of astrocytic mitochondria increased cell death in co-cultured neurons and enhanced changes in mitochondrial membrane potential in astrocytes suggesting a two-way crosstalk between these cells after injury related to energy supply and demand (Voloboueva et al., 2007).

#### **4.4 The role of Grp78/BiP in calcium handling**

The endoplasmic reticulum (ER) controls several cellular processes including protein synthesis, folding and trafficking. Under conditions of physiologic stress, including ischemia, that perturb ER  $\text{Ca}^{2+}$  homeostasis and therefore ER protein folding, the concerted actions of multiple pathways that influence protein synthesis and folding are activated; this is termed the unfolded protein response (UPR). Grp78/BiP is an ER chaperone protein involved in protein folding, suppression of apoptosis and regulation of the UPR. It is strongly induced as part of the UPR and translocates to mitochondria and other compartments after stress where it is postulated to mediate ER-mitochondria crosstalk (Sun et al., 2006a). Several prior studies also supported a role for Grp78/BiP in protection from ischemia-induced cell death using BIX, a Grp78 inducer, prior to transient global forebrain ischemia in gerbils (Oida et al., 2008) and focal ischemia in mice (Kudo et al., 2007). Work from our laboratory further showed that overexpression of Grp78/BiP protected cultured astrocytes from OGD, suppressed the GD-induced increase in mitochondrial  $\text{Ca}^{2+}$  and preserved mitochondrial function (Ouyang et al., 2011).

### **5. Conclusion**

The processes leading to neuronal death following ischemia are complex and involve the integrated action of multiple pathways in a variety of cells types. Data from our laboratory, among others, has highlighted a role for dysfunction of astrocytes and microglia in the pathophysiology of cerebral ischemia. Currently, the most promising areas for intervention are ischemia-induced inflammation and oxidative stress with several drugs in clinical trials at this time aimed at suppressing cytokine release and reactive oxygen species, respectively. For example, the microglial inhibitor minocycline, which affects the release of inflammatory mediators from activated microglia is in Phase IV trials (Yenari et al., 2006), and epoetin alfa, which may be downregulated in astrocytes after ischemic injury is in Phase II/III trials (Zhao and Rempe, 2010). In this chapter we have reviewed several key functions of glial cells including control of inflammation, apoptosis and synaptic glutamate clearance as well as modulation of blood flow and mitochondrial protection (see Figure 1) that may be therapeutically targeted to protect neurons from injury. As the roles of glial cells and heat shock proteins in normal function and cerebral ischemia continue to be elucidated novel neuroprotective strategies may be developed in the future.

Astrocytes are well poised to respond to changes in blood flow by release of vasodilators such as cyclooxygenase (COX) and adenosine. In the case of ischemia from thrombus or embolus, the decrease in oxygen and glucose delivery can initiate a stress response in astrocytes including changes in morphology, increase in intermediate filaments such as GFAP (not shown), decreases in glutamate transporters and activation of mitochondrial cell death pathways. Heat shock proteins have been shown to modulate several of these

pathways to inhibit astrocyte dysfunction leading to neuronal death. Microglia play an important role in the inflammatory cascade following ischemia. Activation of NFκB leads to the production of pro-inflammatory cytokines which can exacerbate damage to neurons. Heat shock proteins may also have a role in inhibiting the activation of NFκB, by direct interaction and stabilization of the IκB:NFκB complex or by inhibition of IKK preventing phosphorylation of degradation of IκB.

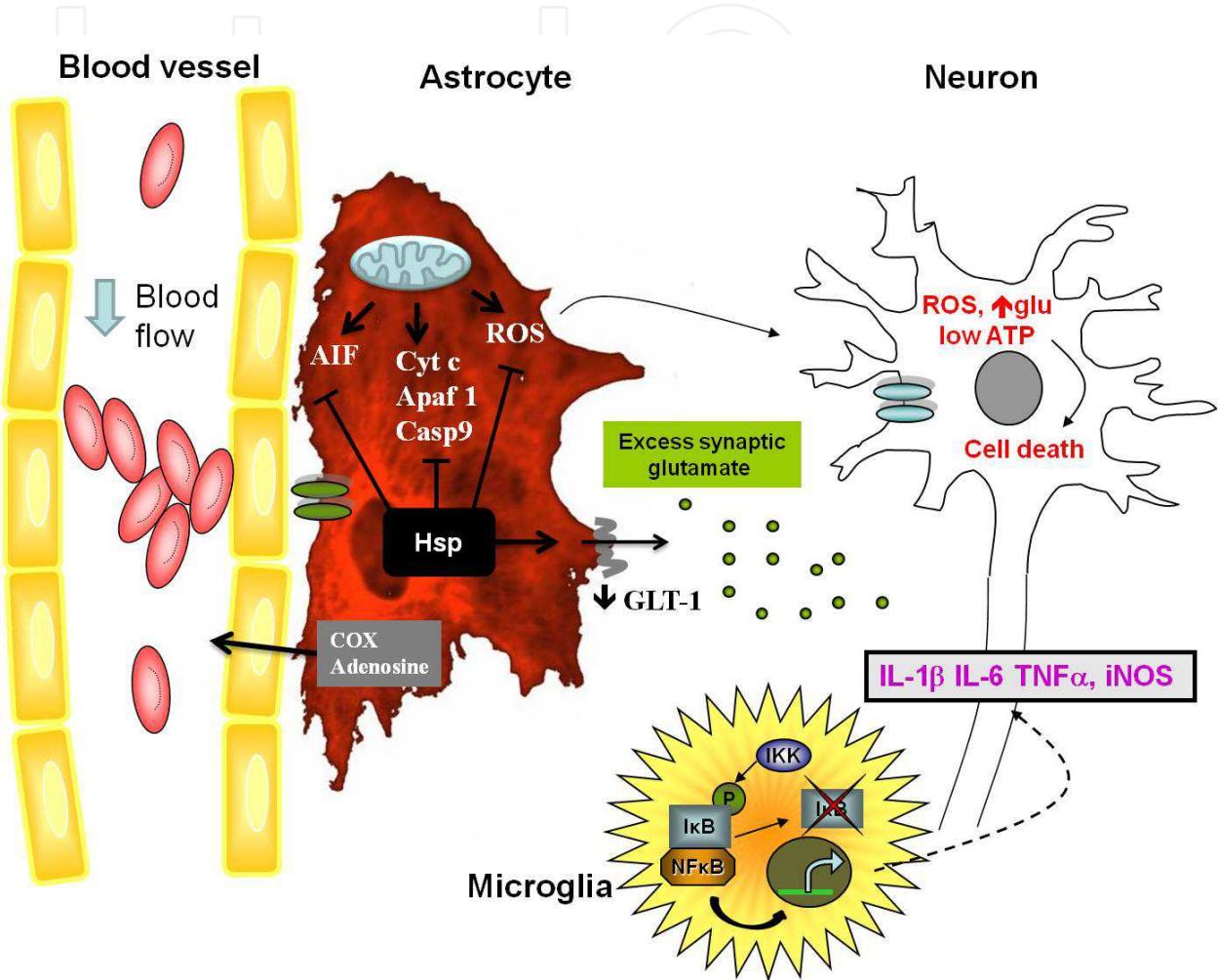


Fig. 1. Glial involvement in neuronal death from ischemia.

## 6. Acknowledgment

This work was supported in part by NIH grants NS053898 and GM49831 to RGG and T32 GM089626 to REW.

## 7. References

- Aloisi, F. (2001). Immune function of microglia. *Glia*, Vol. 36, No. 2, pp. 165-179
- Babcock, A. A., Kuziel, W. A., Rivest, S. & Owens, T. (2003). Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J Neurosci*, Vol. 23, No. 21, pp. 7922-7930

- Bambrick, L., Kristian, T. & Fiskum, G. (2004). Astrocyte mitochondrial mechanisms of ischemic brain injury and neuroprotection. *Neurochem Res*, Vol. 29, No. 3, pp. 601-608
- Barone, F. C., Arvin, B., White, R. F., Miller, A., Webb, C. L., Willette, R. N., Lysko, P. G. & Feuerstein, G. Z. (1997). Tumor Necrosis Factor- $\alpha$  : A Mediator of Focal Ischemic Brain Injury. *Stroke*, Vol. 28, No. 6, pp. 1233-1244
- Bergeron, M., Mivechi, N. F., Giaccia, A. J. & Giffard, R. G. (1996). Mechanism of heat shock protein 72 induction in primary cultured astrocytes after oxygen-glucose deprivation. *Neurol Res*, Vol. 18, No. 1, pp. 64-72
- Bernard, S. A., Gray, T. W., Buist, M. D., Jones, B. M., Silvester, W., Gutteridge, G. & Smith, K. (2002). Treatment of Comatose Survivors of Out-of-Hospital Cardiac Arrest with Induced Hypothermia. *New England Journal of Medicine*, Vol. 346, No. 8, pp. 557-563
- Bush, T. G., Puvanachandra, N., Horner, C. H., Polito, A., Ostensfeld, T., Svendsen, C. N., Mucke, L., Johnson, M. H. & Sofroniew, M. V. (1999). Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron*, Vol. 23, No. 2, pp. 297-308
- Bushong, E. A., Martone, M. E. & Ellisman, M. H. (2004). Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. *Int J Dev Neurosci*, Vol. 22, No. 2, pp. 73-86
- Bushong, E. A., Martone, M. E., Jones, Y. Z. & Ellisman, M. H. (2002). Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci*, Vol. 22, No. 1, pp. 183-192
- Cahoy, J. D., Emery, B., Kaushal, A., Foo, L. C., Zamanian, J. L., Christopherson, K. S., Xing, Y., Lubischer, J. L., Krieg, P. A., Krupenko, S. A., Thompson, W. J. & Barres, B. A. (2008). A Transcriptome Database for Astrocytes, Neurons, and Oligodendrocytes: A New Resource for Understanding Brain Development and Function. *The Journal of Neuroscience*, Vol. 28, No. 1, pp. 264-278
- Chan, P. H. (2004). Mitochondria and neuronal death/survival signaling pathways in cerebral ischemia. *Neurochem Res*, Vol. 29, No. 11, pp. 1943-1949
- Chao, T. I., Rickmann, M. & Wolff, J. R. (2002). The synapse-astrocyte boundary: an anatomical basis for an integrative role of glia in synaptic transmission. In: *The Tripartite Synapse: glia in synaptic transmission*, A. Volterra, P. Magistretti and P. Haydon, Eds, Oxford University Press, Oxford, pp. 3-23.
- Chen, J. C., Hsu-Chou, H., Lu, J. L., Chiang, Y. C., Huang, H. M., Wang, H. L., Wu, T., Liao, J. J. & Yeh, T. S. (2005). Down-regulation of the glial glutamate transporter GLT-1 in rat hippocampus and striatum and its modulation by a group III metabotropic glutamate receptor antagonist following transient global forebrain ischemia. *Neuropharmacology*, Vol. 49, No. 5, pp. 703-714
- Chu, K., Lee, S. T., Sinn, D. I., Ko, S. Y., Kim, E. H., Kim, J. M., Kim, S. J., Park, D. K., Jung, K. H., Song, E. C., Lee, S. K., Kim, M. & Roh, J. K. (2007). Pharmacological Induction of Ischemic Tolerance by Glutamate Transporter-1 (EAAT2) Upregulation. *Stroke*, Vol. 38, No. 1, pp. 177-182
- Cotrina, M. L., Kang, J., Lin, J. H., Bueno, E., Hansen, T. W., He, L., Liu, Y. & Nedergaard, M. (1998). Astrocytic gap junctions remain open during ischemic conditions. *J Neurosci*, Vol. 18, No. 7, pp. 2520-2537
- Danbolt, N. C. (2001). Glutamate uptake. *Prog Neurobiol*, Vol. 65, No. 1, pp. 1-105

- Daneman, R., Zhou, L., Kebede, A. A. & Barres, B. A. (2010). Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature*, Vol. 468, No. 7323, pp. 562-566
- De Bilbao, F., Arsenijevic, D., Moll, T., Garcia-Gabay, I., Vallet, P., Langhans, W. & Giannakopoulos, P. (2009). In vivo over-expression of interleukin-10 increases resistance to focal brain ischemia in mice. *Journal of Neurochemistry*, Vol. 110, No. 1, pp. 12-22
- de Vreede-Swagemakers, J. J., Gorgels, A. P., Dubois-Arbouw, W. I., van Ree, J. W., Daemen, M. J., Houben, L. G. & Wellens, H. J. (1997). Out-of-hospital cardiac arrest in the 1990's: a population-based study in the Maastricht area on incidence, characteristics and survival. *J Am Coll Cardiol*, Vol. 30, No. 6, pp. 1500-1505
- Denes, A., Vidyasagar, R., Feng, J., Narvainen, J., McColl, B. W., Kauppinen, R. A. & Allan, S. M. (2007). Proliferating resident microglia after focal cerebral ischaemia in mice. *J Cereb Blood Flow Metab*, Vol. 27, No. 12, pp. 1941-1953
- Doyle, K. P., Simon, R. P. & Stenzel-Poore, M. P. (2008). Mechanisms of ischemic brain damage. *Neuropharmacology*, Vol. 55, No. 3, pp. 310-318
- Dugan, L. L. & Kim-Han, J. S. (2004). Astrocyte mitochondria in in vitro models of ischemia. *J Bioenerg Biomembr*, Vol. 36, No. 4, pp. 317-321
- Eckstein, M., Stratton, S. J. & Chan, L. S. (2005). Cardiac Arrest Resuscitation Evaluation in Los Angeles: CARE-LA. *Ann Emerg Med*, Vol. 45, No. 5, pp. 504-509
- Eliasson, C., Sahlgren, C., Berthold, C. H., Stakeberg, J., Celis, J. E., Betsholtz, C., Eriksson, J. E. & Pekny, M. (1999). Intermediate filament protein partnership in astrocytes. *J Biol Chem*, Vol. 274, No. 34, pp. 23996-24006
- Eng, L. F., Ghirnikar, R. S. & Lee, Y. L. (2000). Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res*, Vol. 25, No. 9-10, pp. 1439-1451
- Feinstein, D. L., Galea, E., Aquino, D. A., Li, G. C., Xu, H. & Reis, D. J. (1996). Heat Shock Protein 70 Suppresses Astroglial-inducible Nitric-oxide Synthase Expression by Decreasing NFkB Activation. *Journal of Biological Chemistry*, Vol. 271, No. 30, pp. 17724-17732
- Freeman, B. C., Myers, M. P., Schumacher, R. & Morimoto, R. I. (1995). Identification of a regulatory motif in Hsp70 that affects ATPase activity, substrate binding and interaction with HDJ-1. *EMBO J*, Vol. 14, No. 10, pp. 2281-2292
- Gegelashvili, G. & Schousboe, A. (1997). High affinity glutamate transporters: regulation of expression and activity. *Mol Pharmacol*, Vol. 52, No. 1, pp. 6-15
- Gerritse, K., Laman, J. D., Noelle, R. J., Aruffo, A., Ledbetter, J. A., Boersma, W. J. & Claassen, E. (1996). CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci U S A*, Vol. 93, No. 6, pp. 2499-2504
- Giffard, R. G., Han, R. Q., Emery, J. F., Duan, M. & Pittet, J. F. (2008). Regulation of apoptotic and inflammatory cell signaling in cerebral ischemia: the complex roles of heat shock protein 70. *Anesthesiology*, Vol. 109, No. 2, pp. 339-348
- Giffard, R. G. & Yenari, M. A. (2004). Many mechanisms for hsp70 protection from cerebral ischemia. *J Neurosurg Anesthesiol*, Vol. 16, No. 1, pp. 53-61
- Hadari, Y. R., Haring, H. U. & Zick, Y. (1997). p75, a member of the heat shock protein family, undergoes tyrosine phosphorylation in response to oxidative stress. *J Biol Chem*, Vol. 272, No. 1, pp. 657-662
- Hartl, F. U. (1996). Molecular chaperones in cellular protein folding. *Nature*, Vol. 381, No. 6583, pp. 571-579



- Hoehn, B., Ringer, T. M., Xu, L., Giffard, R. G., Sapolsky, R. M., Steinberg, G. K. & Yenari, M. A. (2001). Overexpression of HSP72 after induction of experimental stroke protects neurons from ischemic damage. *J Cereb Blood Flow Metab*, Vol. 21, No. 11, pp. 1303-1309
- Hyakkoku, K., Hamanaka, J., Tsuruma, K., Shimazawa, M., Tanaka, H., Uematsu, S., Akira, S., Inagaki, N., Nagai, H. & Hara, H. (2010). Toll-like receptor 4 (TLR4), but not TLR3 or TLR9, knock-out mice have neuroprotective effects against focal cerebral ischemia. *Neuroscience*, Vol. 171, No. 1, pp. 258-267
- Iadecola, C. & Nedergaard, M. (2007). Glial regulation of the cerebral microvasculature. *Nat Neurosci*, Vol. 10, No. 11, pp. 1369-1376
- Jo, S. K., Ko, G. J., Boo, C. S., Cho, W. Y. & Kim, H. K. (2006). Heat preconditioning attenuates renal injury in ischemic ARF in rats: role of heat-shock protein 70 on NF-kappaB-mediated inflammation and on tubular cell injury. *J Am Soc Nephrol*, Vol. 17, No. 11, pp. 3082-3092
- Kettenmann, H. & Steinhauser, C. (2005). Receptors for neurotransmitters and hormones. In: *Neuroglia*, H. Kettenmann and B. R. Ransom, Eds, Oxford University Press, Oxford, pp. 131-145.
- Kielian, T. (2008). Glial connexins and gap junctions in CNS inflammation and disease. *Journal of Neurochemistry*, Vol. 106, No. 3, pp. 1000-1016
- Kimelberg, H. K. (2004). The problem of astrocyte identity. *Neurochem Int*, Vol. 45, No. 2-3, pp. 191-202
- Kreutzberg, G. W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci*, Vol. 19, No. 8, pp. 312-318
- Kudo, T., Kanemoto, S., Hara, H., Morimoto, N., Morihara, T., Kimura, R., Tabira, T., Imaizumi, K. & Takeda, M. (2007). A molecular chaperone inducer protects neurons from ER stress. *Cell Death Differ*, Vol. 15, No. 2, pp. 364-375
- Lee, A. S. (2001). The glucose-regulated proteins: stress induction and clinical applications. *Trends Biochem Sci*, Vol. 26, No. 8, pp. 504-510
- Lee, S. H., Kim, M., Yoon, B. W., Kim, Y. J., Ma, S. J., Roh, J. K., Lee, J. S. & Seo, J. S. (2001). Targeted hsp70.1 disruption increases infarction volume after focal cerebral ischemia in mice. *Stroke*, Vol. 32, No. 12, pp. 2905-2912
- Lee, S. H., Kwon, H. M., Kim, Y. J., Lee, K. M., Kim, M. & Yoon, B. W. (2004). Effects of hsp70.1 gene knockout on the mitochondrial apoptotic pathway after focal cerebral ischemia. *Stroke*, Vol. 35, No. 9, pp. 2195-2199
- Leist, M. & Jaattela, M. (2001). Four deaths and a funeral: from caspases to alternative mechanisms. *Nat Rev Mol Cell Biol*, Vol. 2, No. 8, pp. 589-598
- Liberto, C. M., Albrecht, P. J., Herx, L. M., Yong, V. W. & Levison, S. W. (2004). Pro-regenerative properties of cytokine-activated astrocytes. *J Neurochem*, Vol. 89, No. 5, pp. 1092-1100
- Liu, B. & Hong, J. S. (2003). Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J Pharmacol Exp Ther*, Vol. 304, No. 1, pp. 1-7
- Liu, D., Smith, C. L., Barone, F. C., Ellison, J. A., Lysko, P. G., Li, K. & Simpson, I. A. (1999). Astrocytic demise precedes delayed neuronal death in focal ischemic rat brain. *Brain Res Mol Brain Res*, Vol. 68, No. 1-2, pp. 29-41
- Lloyd-Jones, D., Adams, R. J., Brown, T. M., Carnethon, M., Dai, S., De Simone, G., Ferguson, T. B., Ford, E., Furie, K., Gillespie, C., Go, A., Greenlund, K., Haase, N., Hailpern, S., Ho, P. M., Howard, V., Kissela, B., Kittner, S., Lackland, D., Lisabeth,



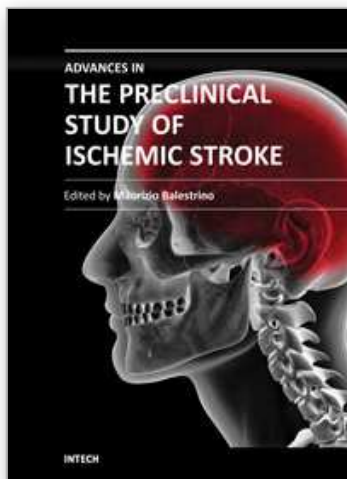
- L., Marelli, A., McDermott, M. M., Meigs, J., Mozaffarian, D., Mussolino, M., Nichol, G., Roger, V. L., Rosamond, W., Sacco, R., Sorlie, P., Thom, T., Wasserthiel-Smoller, S., Wong, N. D. & Wylie-Rosett, J. (2010). Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*, Vol. 121, No. 7, pp. e46-e215
- Lucin, K. M. & Wyss-Coray, T. (2009). Immune Activation in Brain Aging and Neurodegeneration: Too Much or Too Little? *Neuron*, Vol. 64, No. 1, pp. 110-122
- Lukaszewicz, A. C., Sampaio, N., Guegan, C., Benchoua, A., Couriaud, C., Chevalier, E., Sola, B., Lacombe, P. & Onteniente, B. (2002). High sensitivity of protoplasmic cortical astroglia to focal ischemia. *J Cereb Blood Flow Metab*, Vol. 22, No. 3, pp. 289-298
- Maragakis, N. J. & Rothstein, J. D. (2001). Glutamate transporters in neurologic disease. *Arch Neurol*, Vol. 58, No. 3, pp. 365-370
- Massa, S. M., Longo, F. M., Zuo, J., Wang, S., Chen, J. & Sharp, F. R. (1995). Cloning of rat grp75, an hsp70-family member, and its expression in normal and ischemic brain. *J Neurosci Res*, Vol. 40, No. 6, pp. 807-819
- Mitani, A., Andou, Y., Matsuda, S., Arai, T., Sakanaka, M. & Kataoka, K. (1994). Origin of ischemia-induced glutamate efflux in the CA1 field of the gerbil hippocampus: an in vivo brain microdialysis study. *J Neurochem*, Vol. 63, No. 6, pp. 2152-2164
- Mitani, A. & Tanaka, K. (2003). Functional Changes of Glial Glutamate Transporter GLT-1 during Ischemia: An In Vivo Study in the Hippocampal CA1 of Normal Mice and Mutant Mice Lacking GLT-1. *The Journal of Neuroscience*, Vol. 23, No. 18, pp. 7176-7182
- Nawashiro, H., Brenner, M., Fukui, S., Shima, K. & Hallenbeck, J. M. (2000). High susceptibility to cerebral ischemia in GFAP-null mice. *J Cereb Blood Flow Metab*, Vol. 20, No. 7, pp. 1040-1044
- Oida, Y., Izuta, H., Oyagi, A., Shimazawa, M., Kudo, T., Imaizumi, K. & Hara, H. (2008). Induction of BiP, an ER-resident protein, prevents the neuronal death induced by transient forebrain ischemia in gerbil. *Brain Research*, Vol. 1208, No. 217-224
- Ouyang, Y. B., Voloboueva, L. A., Xu, L. J. & Giffard, R. G. (2007). Selective dysfunction of hippocampal CA1 astrocytes contributes to delayed neuronal damage after transient forebrain ischemia. *J Neurosci*, Vol. 27, No. 16, pp. 4253-4260
- Ouyang, Y. B., Xu, L. J., Emery, J. F., Lee, A. S. & Giffard, R. G. (2011). Overexpressing GRP78 influences Ca<sup>2+</sup> handling and function of mitochondria in astrocytes after ischemia-like stress. *Mitochondrion*, Vol. 11, No. 2, pp. 279-286
- Ouyang, Y. B., Xu, L. J., Sun, Y. J. & Giffard, R. G. (2006). Overexpression of inducible heat shock protein 70 and its mutants in astrocytes is associated with maintenance of mitochondrial physiology during glucose deprivation stress. *Cell Stress Chaperones*, Vol. 11, No. 2, pp. 180-186
- Papadopoulos, M. C., Sun, X. Y., Cao, J., Mivechi, N. F. & Giffard, R. G. (1996). Overexpression of HSP-70 protects astrocytes from combined oxygen-glucose deprivation. *Neuroreport*, Vol. 7, No. 2, pp. 429-432
- Pekny, M., Leveen, P., Pekna, M., Eliasson, C., Berthold, C. H., Westermarck, B. & Betsholtz, C. (1995). Mice lacking glial fibrillary acidic protein display astrocytes devoid of intermediate filaments but develop and reproduce normally. *EMBO J*, Vol. 14, No. 8, pp. 1590-1598
- Pekny, M. & Nilsson, M. (2005). Astrocyte activation and reactive gliosis. *Glia*, Vol. 50, No. 4, pp. 427-434

- Pekny, M. & Pekna, M. (2004). Astrocyte intermediate filaments in CNS pathologies and regeneration. *J Pathol*, Vol. 204, No. 4, pp. 428-437
- Pellerin, L. (2005). How astrocytes feed hungry neurons. *Mol Neurobiol*, Vol. 32, No. 1, pp. 59-72
- Petito, C. K., Olarte, J. P., Roberts, B., Nowak, T. S., Jr. & Pulsinelli, W. A. (1998). Selective glial vulnerability following transient global ischemia in rat brain. *J Neuropathol Exp Neurol*, Vol. 57, No. 3, pp. 231-238
- Pulsinelli, W. A. (1985). Selective Neuronal Vulnerability: Morphological and Molecular Characteristics. In: *Progress in Brain Research*, K. A. H. B. K. S. K. Kogure and F. A. Welsh, Eds, Vol. Volume 63, Elsevier, pp. 29-37.
- Rajdev, S., Hara, K., Kokubo, Y., Mestril, R., Dillmann, W., Weinstein, P. R. & Sharp, F. R. (2000). Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. *Ann Neurol*, Vol. 47, No. 6, pp. 782-791
- Ran, R., Lu, A., Zhang, L., Tang, Y., Zhu, H., Xu, H., Feng, Y., Han, C., Zhou, G., Rigby, A. C. & Sharp, F. R. (2004). Hsp70 promotes TNF-mediated apoptosis by binding IKK $\gamma$  and impairing NF- $\kappa$ B survival signaling. *Genes & Development*, Vol. 18, No. 12, pp. 1466-1481
- Rao, V. L., Bowen, K. K. & Dempsey, R. J. (2001a). Transient focal cerebral ischemia down-regulates glutamate transporters GLT-1 and EAAC1 expression in rat brain. *Neurochem Res*, Vol. 26, No. 5, pp. 497-502
- Rao, V. L., Dogan, A., Todd, K. G., Bowen, K. K., Kim, B. T., Rothstein, J. D. & Dempsey, R. J. (2001b). Antisense knockdown of the glial glutamate transporter GLT-1, but not the neuronal glutamate transporter EAAC1, exacerbates transient focal cerebral ischemia-induced neuronal damage in rat brain. *J Neurosci*, Vol. 21, No. 6, pp. 1876-1883
- Rao, V. L. R., Rao, A. M., Dogan, A., Bowen, K. K., Hatcher, J., Rothstein, J. D. & Dempsey, R. J. (2000). Glial glutamate transporter GLT-1 down-regulation precedes delayed neuronal death in gerbil hippocampus following transient global cerebral ischemia. *Neurochem Int*, Vol. 36, No. 6, pp. 531-537
- Ravagnan, L., Gurbuxani, S., Susin, S. A., Maise, C., Daugas, E., Zamzami, N., Mak, T., Jaattela, M., Penninger, J. M., Garrido, C. & Kroemer, G. (2001). Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nat Cell Biol*, Vol. 3, No. 9, pp. 839-843
- Reichenbach, A. & Wolburg, H. (2005). Astrocytes and ependymal glia. In: *Neuroglia*, H. Kettenmann and B. R. Ransom, Eds, Oxford University Press, Oxford, pp. 19-35.
- Rosenberg, P. A. & Aizenman, E. (1989). Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte-poor cultures of rat cerebral cortex. *Neurosci Lett*, Vol. 103, No. 2, pp. 162-168
- Rothstein, J. D., Dykes-Hoberg, M., Pardo, C. A., Bristol, L. A., Jin, L., Kuncl, R. W., Kanai, Y., Hediger, M. A., Wang, Y., Schielke, J. P. & Welty, D. F. (1996). Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron*, Vol. 16, No. 3, pp. 675-686
- Ryan, A. J., Flanagan, S. W., Moseley, P. L. & Gisolfi, C. V. (1992). Acute heat stress protects rats against endotoxin shock. *J Appl Physiol*, Vol. 73, No. 4, pp. 1517-1522
- Sahota, P. & Savitz, S. I. (2011). Investigational Therapies for Ischemic Stroke: Neuroprotection and Neurorecovery. *Neurotherapeutics*, Vol. 8, No. 3, pp. 434-451
- Schmid, R. S., Yokota, Y. & Anton, E. S. (2006). Generation and characterization of brain lipid-binding protein promoter-based transgenic mouse models for the study of radial glia. *Glia*, Vol. 53, No. 4, pp. 345-351

- Seki, Y., Feustel, P. J., Keller, R. W., Jr., Tranmer, B. I. & Kimelberg, H. K. (1999). Inhibition of ischemia-induced glutamate release in rat striatum by dihydrokinate and an anion channel blocker. *Stroke*, Vol. 30, No. 2, pp. 433-440
- Singleton, K. D. & Wischmeyer, P. E. (2006). Effects of HSP70.1/3 gene knockout on acute respiratory distress syndrome and the inflammatory response following sepsis. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, Vol. 290, No. 5, pp. L956-L961
- Soriano, M. A., Planas, A. M., Rodríguez-Farré, E. & Ferrer, I. (1994). Early 72-kDa heat shock protein induction in microglial cells following focal ischemia in the rat brain. *Neuroscience Letters*, Vol. 182, No. 2, pp. 205-207
- Sun, F.-C., Wei, S., Li, C.-W., Chang, Y.-S., Chao, C.-C. & Lai, Y.-K. (2006a). Localization of GRP78 to mitochondria under the unfolded protein response. *Biochem J*, Vol. 396, No. 1, pp. 31-39
- Sun, Y., Ouyang, Y. B., Xu, L., Chow, A. M., Anderson, R., Hecker, J. G. & Giffard, R. G. (2006b). The carboxyl-terminal domain of inducible Hsp70 protects from ischemic injury in vivo and in vitro. *J Cereb Blood Flow Metab*, Vol. 26, No. 7, pp. 937-950
- Sweitzer, S. M., Hickey, W. F., Rutkowski, M. D., Pahl, J. L. & DeLeo, J. A. (2002). Focal peripheral nerve injury induces leukocyte trafficking into the central nervous system: potential relationship to neuropathic pain. *Pain*, Vol. 100, No. 1-2, pp. 163-170
- Takano, T., Tian, G. F., Peng, W., Lou, N., Libionka, W., Han, X. & Nedergaard, M. (2006). Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci*, Vol. 9, No. 2, pp. 260-267
- Tanaka, K., Watase, K., Manabe, T., Yamada, K., Watanabe, M., Takahashi, K., Iwama, H., Nishikawa, T., Ichihara, N., Kikuchi, T., Okuyama, S., Kawashima, N., Hori, S., Takimoto, M. & Wada, K. (1997). Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science*, Vol. 276, No. 5319, pp. 1699-1702
- Tanaka, R., Komine-Kobayashi, M., Mochizuki, H., Yamada, M., Furuya, T., Migita, M., Shimada, T., Mizuno, Y. & Urabe, T. (2003). Migration of enhanced green fluorescent protein expressing bone marrow-derived microglia/macrophage into the mouse brain following permanent focal ischemia. *Neuroscience*, Vol. 117, No. 3, pp. 531-539
- The Hypothermia after Cardiac Arrest Study Group. (2002). Mild Therapeutic Hypothermia to Improve the Neurologic Outcome after Cardiac Arrest. *New England Journal of Medicine*, Vol. 346, No. 8, pp. 549-556
- The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. (1995). Tissue Plasminogen Activator for Acute Ischemic Stroke. *New England Journal of Medicine*, Vol. 333, No. 24, pp. 1581-1588
- van der Weerd, L., Lythgoe, M. F., Badin, R. A., Valentim, L. M., Akbar, M. T., de Belleruche, J. S., Latchman, D. S. & Gadian, D. G. (2005). Neuroprotective effects of HSP70 overexpression after cerebral ischaemia--An MRI study. *Experimental Neurology*, Vol. 195, No. 1, pp. 257-266
- Vibulsreth, S., Hefti, F., Ginsberg, M. D., Dietrich, W. D. & Busto, R. (1987). Astrocytes protect cultured neurons from degeneration induced by anoxia. *Brain Research*, Vol. 422, No. 2, pp. 303-311
- Villar, J., Ribeiro, S. P., Mullen, J. B., Kuliszewski, M., Post, M. & Slutsky, A. S. (1994). Induction of the heat shock response reduces mortality rate and organ damage in a sepsis-induced acute lung injury model. *Crit Care Med*, Vol. 22, No. 6, pp. 914-921

- Voloboueva, L. A., Duan, M., Ouyang, Y., Emery, J. F., Stoy, C. & Giffard, R. G. (2008). Overexpression of mitochondrial Hsp70/Hsp75 protects astrocytes against ischemic injury in vitro. *J Cereb Blood Flow Metab*, Vol. 28, No. 5, pp. 1009-1016
- Voloboueva, L. A., Suh, S. W., Swanson, R. A. & Giffard, R. G. (2007). Inhibition of mitochondrial function in astrocytes: implications for neuroprotection. *J Neurochem*, Vol. 102, No. 4, pp. 1383-1394
- Voos, W., Martin, H., Krimmer, T. & Pfanner, N. (1999). Mechanisms of protein translocation into mitochondria. *Biochim Biophys Acta*, Vol. 1422, No. 3, pp. 235-254
- Weiss, Y. G., Maloyan, A., Tazelaar, J., Raj, N. & Deutschman, C. S. (2002). Adenoviral transfer of HSP-70 into pulmonary epithelium ameliorates experimental acute respiratory distress syndrome. *The Journal of Clinical Investigation*, Vol. 110, No. 6, pp. 801-806
- Wilhelmsson, U., Li, L., Pekna, M., Berthold, C. H., Blom, S., Eliasson, C., Renner, O., Bushong, E., Ellisman, M., Morgan, T. E. & Pekny, M. (2004). Absence of glial fibrillary acidic protein and vimentin prevents hypertrophy of astrocytic processes and improves post-traumatic regeneration. *J Neurosci*, Vol. 24, No. 21, pp. 5016-5021
- Xu, L., Emery, J. F., Ouyang, Y. B., Voloboueva, L. A. & Giffard, R. G. (2010). Astrocyte targeted overexpression of Hsp72 or SOD2 reduces neuronal vulnerability to forebrain ischemia. *Glia*, Vol. 58, No. 9, pp. 1042-1049
- Xu, L. & Giffard, R. G. (1997). HSP70 protects murine astrocytes from glucose deprivation injury. *Neurosci Lett*, Vol. 224, No. 1, pp. 9-12
- Xu, L., Lee, J. E. & Giffard, R. G. (1999). Overexpression of bcl-2, bcl-XL or hsp70 in murine cortical astrocytes reduces injury of co-cultured neurons. *Neurosci Lett*, Vol. 277, No. 3, pp. 193-197
- Xu, L., Sapolsky, R. M. & Giffard, R. G. (2001). Differential sensitivity of murine astrocytes and neurons from different brain regions to injury. *Exp Neurol*, Vol. 169, No. 2, pp. 416-424
- Xu, L., Voloboueva, L. A., Ouyang, Y., Emery, J. F. & Giffard, R. G. (2009). Overexpression of mitochondrial Hsp70/Hsp75 in rat brain protects mitochondria, reduces oxidative stress, and protects from focal ischemia. *J Cereb Blood Flow Metab*, Vol. 29, No. 2, pp. 365-374
- Yeh, T. H., Hwang, H. M., Chen, J. J., Wu, T., Li, A. H. & Wang, H. L. (2005). Glutamate transporter function of rat hippocampal astrocytes is impaired following the global ischemia. *Neurobiol Dis*, Vol. 18, No. 3, pp. 476-483
- Yenari, M. A., Kauppinen, T. M. & Swanson, R. A. (2010). Microglial activation in stroke: therapeutic targets. *Neurotherapeutics*, Vol. 7, No. 4, pp. 378-391
- Yenari, M. A., Xu, L., Tang, X. N., Qiao, Y. & Giffard, R. G. (2006). Microglia potentiate damage to blood-brain barrier constituents: improvement by minocycline in vivo and in vitro. *Stroke*, Vol. 37, No. 4, pp. 1087-1093
- Yu, A. C., Gregory, G. A. & Chan, P. H. (1989). Hypoxia-induced dysfunctions and injury of astrocytes in primary cell cultures. *J Cereb Blood Flow Metab*, Vol. 9, No. 1, pp. 20-28
- Zhao, X., Ahram, A., Berman, R. F., Muizelaar, J. P. & Lyeth, B. G. (2003). Early loss of astrocytes after experimental traumatic brain injury. *Glia*, Vol. 44, No. 2, pp. 140-152
- Zhao, Y. & Rempe, D. A. (2010). Targeting astrocytes for stroke therapy. *Neurotherapeutics*, Vol. 7, No. 4, pp. 439-451
- Zheng, Z., Kim, J. Y., Ma, H., Lee, J. E. & Yenari, M. A. (2008). Anti-inflammatory effects of the 70 kDa heat shock protein in experimental stroke. *J Cereb Blood Flow Metab*, Vol. 28, No. 1, pp. 53-63





## **Advances in the Preclinical Study of Ischemic Stroke**

Edited by Dr. Maurizio Balestrino

ISBN 978-953-51-0290-8

Hard cover, 530 pages

**Publisher** InTech

**Published online** 16, March, 2012

**Published in print edition** March, 2012

This book reports innovations in the preclinical study of stroke, including - novel tools and findings in animal models of stroke, - novel biochemical mechanisms through which ischemic damage may be both generated and limited, - novel pathways to neuroprotection. Although hypothermia has been so far the sole "neuroprotection" treatment that has survived the translation from preclinical to clinical studies, progress in both preclinical studies and in the design of clinical trials will hopefully provide more and better treatments for ischemic stroke. This book aims at providing the preclinical scientist with innovative knowledge and tools to investigate novel mechanisms of, and treatments for, ischemic brain damage.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Vivianne L. Tawfik, Robin E. White and Rona Giffard (2012). Glial Cells, Inflammation and Heat Shock Proteins in Cerebral Ischemia, *Advances in the Preclinical Study of Ischemic Stroke*, Dr. Maurizio Balestrino (Ed.), ISBN: 978-953-51-0290-8, InTech, Available from: <http://www.intechopen.com/books/advances-in-the-preclinical-study-of-ischemic-stroke/glia-cells-inflammation-and-heat-shock-proteins-in-cerebral-ischemia>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen