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

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## 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 LXSN-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 NF $\kappa$ B 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 NF $\kappa$ B, by direct interaction and stabilization of the I $\kappa$ B:NF $\kappa$ B complex or by inhibition of IKK preventing phosphorylation and degradation of I $\kappa$ B.

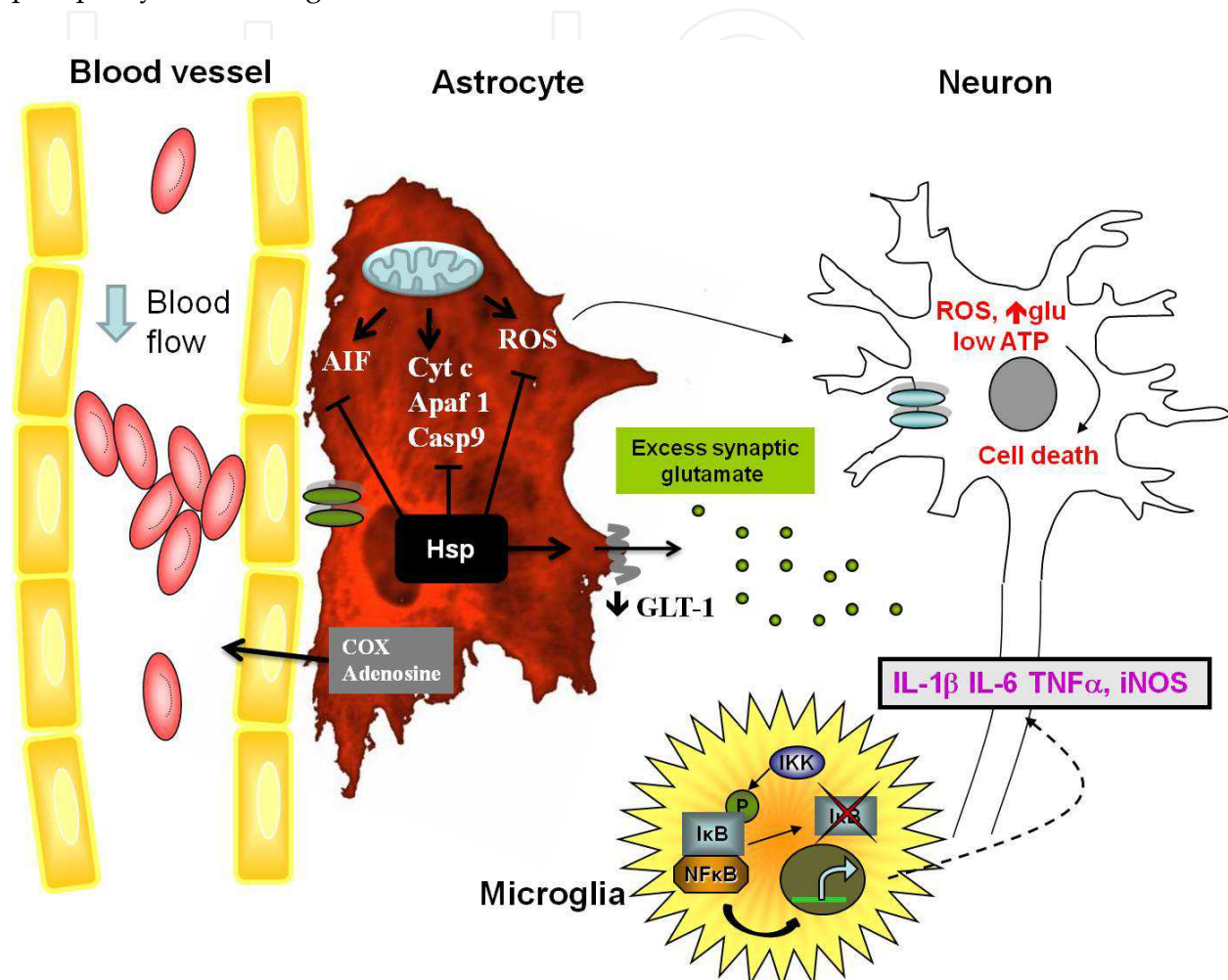


Fig. 1. Glial involvement in neuronal death from ischemia.

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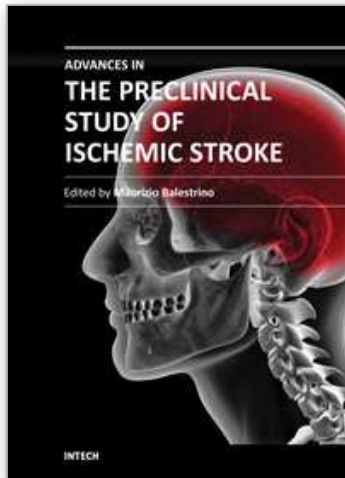
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This book reports innovations in the preclinical study of stroke, including - novel tools and findings in animal models of stroke, - novel biochemical mechanisms through which ischemic damage may be both generated and limited, - novel pathways to neuroprotection. 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.

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