Cerebral ischemia-Reperfusion Injury

1.0 Cerebral Ischemic Disease

Cerebral ischemic disease is characterized by rapid loss of brain function due to disturbances in the blood supply to the brain caused by thrombosis, arterial embolism/spasm or haemorrhage (Marteau et al. 2012). Ischemia of cerebral tissue and cellular death underline all forms of stroke, including focal ischemia (as in occlusion of the middle cerebral artery), global ischemia (as in occlusion of the common carotid arteries) and likely intraparenchymal hemorrhage (Traystman et al. 2003). Prolonged ischemia leads to cell death. In cerebral ischemia, restoration of blood flow can be achieved by cerebral revascularization, using thrombolytics or bypass surgery. However, reperfusion may exacerbate the injury initially caused by ischemia, producing so-called “cerebral reperfusion injury” (Pan et al. 2007). Therefore limiting reperfusion injury was paid further attention in the treatment of cerebral ischemia to avail the complete benefit of reperfusion. The important pathobiological mechanisms of ischemia-reperfusion (I/R) injury include excitotoxicity, oxidative stress, calcium overload, inflammation, blood brain barrier destruction and apoptosis (Kaste 1997; Kim 1997). Recent evidences showed that anti oxidants and anti inflammatory agents may play an important role in limiting reperfusion injury.

1.1 Reperfusion injury

Brain tissue is exquisitely sensitive to ischemic injury; energy production for the maintenance of brain function fails rapidly with the onset of ischemia and is reinstated with timely reperfusion (Pundik et al. 2012). Reperfusion is the goal of most clinical therapies for cerebral ischemic injury. Unfortunately, the benefits
of reperfusion are mitigated by secondary injury to the penumbra, which is in part, related to acute and prolonged inflammatory events. This secondary injury is called ischemia-reperfusion injury. Cerebral ischemia-reperfusion injury is severe and causes mortality and long lasting disability in adults across the globe. Ischemia-reperfusion occurs in various clinical conditions relevant to adults, including myocardial infarction, stroke and circulating shock as well as surgical interventions like organ transplantation and cardiopulmonary bypass surgery (Pacher and Hasko 2008).

1.2 Mechanisms involved in cerebral ischemia-reperfusion injury

1.2.1 Excitotoxicity

Glutamate is an excitatory neurotransmitter in brain tissue and plays a key role in modulating the intracellular communication, plasticity, growth and differentiation. Glutamate is responsible for instigation of postsynaptic signaling through distinct ionotropic and metabotropic glutamate receptors. In ischemic conditions the extracellular glutamate concentrations were increased drastically (Lai et al. 2011). Thus glutamate activity was sustained, causing prolonged depolarization of presynaptic vesicles, which is further exacerbated by the inhibition of neurotransmitter reuptake and energy failure (Crawford et al. 2011). Excitotoxic environment in cell was increased due to increased levels of extracellular glutamate and activation of glutamate receptors. Excessive release of glutamate, astrocytic dysfunction and death is accompanied in cerebral ischemia-reperfusion injury (Danilov and Fiskum 2008).

1.2.2 Calcium overload

Intracellular calcium homeostasis is essential for neuronal development and function. In the central nervous system (CNS) calcium influx through voltage
gated calcium channels (VGCC) regulates numerous processes including neuronal growth, differentiation, motility and excitability, secretion of neurotransmitters and hormones, synaptic plasticity, neurotoxicity and neuronal gene expression. The extracellular calcium ([Ca\(^{2+}\)]_e) can enter in to the neurons through voltage-gated Ca\(^{2+}\) channels and transmitter-gated channels permeable to Ca\(^{2+}\). Under normal physiological conditions, the channel within the NMDA receptor is blocked in a voltage-dependent manner by magnesium ion (Mg\(^{2+}\)), which is dislodged from the channel by mild depolarization (Lipton 2004). In acute ischemic condition, persistent depolarization occurs which leads to activation of NMDA receptor, an important route of Ca\(^{2+}\) influx during glutameric transmission (Crawford et al. 2011). AMPA and kainate receptors are generally involved in Na\(^+\) conductance. Besides ionotropic receptors, glutamate also stimulates a family of metabotropic receptors that activate second messenger pathways resulting in the release of [Ca\(^{2+}\)]_i. The deleterious effects of increased cytosolic calcium include generation of free radicals, disruption of mitochondrial function, the activation of neuronal nitric oxide synthetase (nNOS) to form nitric oxide, degradation of cellular lipids by activation of phospholipases and proteases and deterioration of DNA by activation of nucleases (Ankarcrona et al. 1995; Kristian and Siesjo 1998; Blomgren and Hagberg 2006). It is well understood that mitochondrial dysfunction, specifically the mitochondrial outer membrane elicits the release of cytochrome C, activation of caspases 9 and 3, and apoptosis-inducing factor (AIF), which leads to apoptosis (Hagberg et al. 2009). Release of NO further leads to the formation of superoxide (O\(_2^−\)), peroxynitrite (ONOO\(^−\)) and hydroxyl (OH\(^−\)) radicals (Beckman et al. 1990). An important target of NO induced ONOO\(^−\) is mitochondria and mitochondrial dysfunction during severe
hypoxia–ischemia results in increased generation of oxygen free radicals leading to prompt dysfunction of cellular membrane causing necrosis (Beckman et al. 1990).

1.2.3 Oxidative stress

There is also evidence that increased intracellular Ca$^{2+}$ concentration and excitotoxicity are associated with the generation of free radicals and reactive oxygen species (ROS) (Gunasekar et al. 1995; Rego and Oliveira 2003). Reactive nitrogen intermediates (i.e. nitric oxide) and ROS produced by phagocytes are important for defense against the inflammatory response. However, free radicals are usually a component of the defense and immune reaction. These substances toxic effects also cause cellular injury and death in neurons and other cells. Therefore they may promote complications and long-term defects. These are generally released by granulocytes, microglia and endothelial cells. Oxygen and nitrogen radicals promote the formation of the relatively stable peroxynitrite, which has been considered a central mediator of cellular damage. These molecules mediate cell death by membrane peroxidation, breakdown of protein structure, DNA damage and subsequent activation of poly (ADP)-ribose polymerase (PARP) leading to energy depletion and cell death. In cerebral ischemia-reperfusion, increased production of ROS is an important underlying cause for neuronal injury leading to delayed neuronal death (Wang et al. 2006).

1.2.4 Stress signaling in response to cerebral ischemia-reperfusion

Cerebral cells damage/survival managed by multitude stimuli of elaborate cell signaling pathways. In the cerebral ischemia-reperfusion, the activation of intrinsic cell signaling occurs, in that MAPKs mediated cell signaling from intracellular targets and extracellular environment to the nucleus through
phosphorylation and participate in the transduction of cellular response (Son et al. 2011). MAPK signaling plays a noticeable role in regulating cell survival following cerebral ischemia. The stress-activated MAPKs (p38 and JNK) mainly function as mediators of cellular stress including focal and global cerebral ischemia by phosphorylating intracellular enzymes (Son et al. 2011). Cytosolic proteins and transcription factors are involved in inflammatory cytokine production, cell survival and apoptosis following glutamate stimulated NMDA receptor and PSD95–nNOS interaction is involved in neuronal death (Kontny et al. 1999; Kumar et al. 2003; Zhu et al. 2009; Cao et al. 2005).

1.2.5 Inflammation

Cerebral ischemia initiates an inflammatory response that further leads to mitochondrial injury. This response occurs quicker and is more vigorous with reperfusion (Pundik et al. 2012). The inflammatory response results in activation of complement system, platelets and endothelium. Chronological expression of adhesion molecules, including selectins, intercellular adhesion molecules and vascular cell adhesion molecules results in first neutrophil and later monocyte adhesion to the endothelial wall (Winquist and Kerr 1997). Activated leukocytes contribute to releasing of pro-inflammatory cytokines, proteases and ROS, which injure the endothelial surface, leading to thrombus formation, vasospasm and worsening ischemia. Inflammatory mediators contribute to breakdown of the blood–brain barrier, further promoting the infiltration of leukocytes into the brain. Under conditions like inflammation, peroxynitrite, a reactive oxidant produced from nitric oxide (NO) and superoxide reacts with proteins, lipids and DNA (Pundik et al. 2012). The delayed phase of the inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free
radicals and oxidants, as well as the release of other neutrophil-derived mediators (Shimakura et al. 2000).

Adhesion molecules, cytokines and leukocyte chemo attractants released/presented at the site of blood-brain barrier play a prominent role in mobilizing peripheral inflammatory cells into the brain. Cerebral endothelial cells (CEC) are actively engaged in processes of microvascular stasis and leukocyte infiltration by producing a plethora of pro-inflammatory mediators. Pro-inflammatory cytokines invoke a pleiotropic cellular response, including the stimulation of oxygen centered free radicals during inflammation damage. When challenged by external stimuli including cytokines and hypoxia, CEC have been shown to release/express various products of arachidonic acid cascade with both vasoactive and pro-inflammatory properties, including prostaglandins, leukotrienes and platelet-activating factor (PAF). These metabolites induce platelet and neutrophil activation and adhesion, changes in local cerebral blood flow and blood rheology, an increase in BBB permeability (Fernandez-Lopez et al. 2012). Ischemic CEC have also been shown to express and release bioactive inflammatory cytokines and chemokines including IL-1β, IL-8 and MCP-1 (Ebadi et al. 1997).

1.2.6 Destruction of blood brain barrier (BBB)

The BBB is composed of astrocytes, endothelial cells, pericytes, neurons and the extracellular matrix (ECM), which is collectively known as the neurovascular unit (NVU). BBB endothelial cells have tight junctions (TJs), have minimal pinocytotic activity, lack fenestrations and express a number of enzymes capable of degrading both harmful and therapeutic molecules (Ronaldson and Davis 2012). They also have increased mitochondrial content, which is required
for the multiple energy-dependent processes involved in nutrient support and protection of the brain. Pericytes are vascular smooth-muscle-lineage cells that occur as solitary cells embedded in the basement membrane of microvessels and have their own characteristic morphology (Ronaldson and Davis 2012). Both the endothelial cells and pericytes are surrounded by the basal lamina, which is 30 to 40 nm contiguous with the plasma membranes of astrocyte end-feet. Blood-brain barrier disruption is thought to play a critical role in the pathophysiology of ischemia-reperfusion (Fujimura et al. 1999).

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that can degrade all the components of the extracellular matrix when they are activated. Gelatinase A (MMP-2) and gelatinase B (MMP-9) are able to digest the endothelial basal lamina, which plays a major role in maintaining blood–brain barrier (BBB) impermeable damage after acute brain injury (Fujimura et al. 1999). MMP-9 substrates in the BBB, white matter and extracellular matrix damage would play a central role in the pathophysiology of cerebral ischemia-reperfusion injury. The BBB protein is associated with endothelial tight junction formation. It’s degradation is time dependent. In white matter, three of the four MMP isoforms were degraded after ischemia. Cleavage of matrix proteins by activated MMPs increases the permeability of vessels and compromises the blood–brain barrier. Caveolin-1 (cav-1) is regulating protein in blood–brain barrier, down-regulated BBB and the production of nitric oxide induced the loss of cav-1. Furthermore, it is having impact on BBB permeability and MMPs activity. In cerebral ischemia-reperfusion injury, loss of cav-1 and excessive levels of MMPs were found (Liu et al. 2012b).
1.2.7 Renin angiotensin system

Renin angiotensin system (RAS) plays a key role in physiological functions of brain. Angiotensin is an important brain regulatory neuropeptide. The main active principle of RAS is Angiotensin II. It physiologically stimulates the AT1 receptor. Angiotensin II is a multitasking regulatory factor in brain such that regulation of the stress, regulation of the autonomic and hormone systems and regulation of the circulation in the brain. It is also involved in the response of the brain to endogenous and peripheral inflammation (Saavedra et al. 2011). Recent evidences showed that excessive activation of angiotensin II AT1 receptors is involved in pathogenesis of cerebral ischemic reperfusion injury (Liu et al. 2012a; Schulz et al. 2006). Furthermore, they involved in the progression of inflammation process in injured brain tissue. Therefore angiotensin II AT1 receptors blockage may ameliorate stress and inflammation which is caused by ischemia-reperfusion (Saavedra et al. 2011; Liu et al. 2012a).

1.2.8 Protein kinase C

In brain tissues, protein kinase C (PKC) plays a central role in mediating ischemia-reperfusion damage. In particular, delta PKC has been implicated in mediating oxidative stress, inflammation and apoptosis which are hallmarks of reperfusion injury (Lin et al. 2010). Conflicting reports exist on the role of individual PKC isozymes in cerebral ischemic injury. Recent evidences showed that, delta PKC inhibition reduced cellular injury in a rat hippocampal slice model of cerebral ischemia [oxygen-glucose deprivation (OGD)] when present both during OGD and for the first 3 hr of reperfusion (Bright et al. 2004). Deleterious role for delta PKC during reperfusion suggest that deltaV1-1 delivery, even hours
after commencement of reperfusion, may provide a therapeutic advantage after cerebral ischemia (Bright et al. 2004).

1.2.9 Apoptosis

More brain lesions developed in infarction zone due to reduction of CBF in cerebral ischemia, which become necrotic and then spread towards periphery of occluded artery. Invariably a consequent inflammatory response is developed in ischemic condition. Furthermore, reperfusion induces exaggeration of inflammation which may lead to DNA fragmentation in necrotic cells and leads to apoptosis (Li et al. 1995).

1.3 Therapeutic Interventions

1.3.1 Acute Interventions

Global cerebral ischemia-reperfusion leads to cell apoptosis through several complicated molecular pathways. Neuroprotective therapeutic approaches via molecular mechanisms show better results of stroke treatment. Previous researchers suggested number of therapeutic strategies to inhibit or reduce injury via the molecular mechanisms. Anti-apoptotic protein (i.e., bcl-2) has been over-expressed in rats and has provided moderate resistance to cerebral ischemic damage in both focal and global ischemic models (Brambrink et al. 2004; Chen et al. 1995). The over-expression of free radical scavenging enzymes has reduced cerebral ischemic injury (Yang et al. 2012). Use of some NMDA receptor antagonists (MK-801 and remacemide) also shown efficacy in reducing ischemic injury, as well as the application of peptides that disrupt the interaction between the NMDAR with the postsynaptic density protein PSD-95 has been reported to block downstream neurotoxic signaling without impeding essential neuronal excitation (Gill et al. 1991). Na⁺ and Ca²⁺ channel blockers are shown to
have neuroprotection in i.e., tetrodotoxin (SB206284) both focal and global cerebral ischemic rats. (Xie et al. 1994; Wood et al. 1997). Despite the large number of therapeutic interventions that decrease ischemic brain damage in experimental models, they have not translated well into the improvement in clinical setting (Emerich 2000).

1.3.2 Anti inflammatory Interventions

Alterations of the expression and release of inflammatory mediators in the brain is being investigated as a therapeutic approach to reduce ischemic reperfusion brain injury. Anti-inflammatory therapy may be useful as an adjuvant therapy to thrombolytics. Post-ischemic brain inflammation directly or indirectly targeted several therapeutic strategies which are developed and tested in animal and human studies. The two most important approaches are: i) reducing the overall immune response with non-selective immunosuppressants and ii) targeting a single inflammatory mediator. Recent animal studies have shown that cyclosporine A and NSAIDs decrease the brain infarction after global and focal ischemia (Akdemir et al. 2005; Khansari and Halliwell 2009). Targeting immune cells and inflammatory mediators (i.e. leukocyte and endothelial cell adhesion molecules and cytokines) had variable success. Blocking IL-1β, have been reported to be neuroprotective following experimental cerebral ischemia (Hara et al. 1997; Chao et al. 2012). TNFα inhibitor CNI-1493 and anti-TNFα antibody have been reduced brain infarct volumes after MCAO, but anti-TNFα strategies have not been tested in stroke clinical trials (Meistrell et al. 1997; Noelker et al. 2012). Neutrophil depletion and blocking or deficiency of adhesion receptors on either the neutrophil (i.e., CD18, Mac-1) or the endothelial cells (ICAM-1) has also shown neuroprotection in animal models of cerebral ischemia.
(Yang et al. 2011; Cheng et al. 2008). Leukocyte adhesion molecule inhibitors showed to be safe and effective in the adjuvant therapy of stroke. However, in contrast to the protective effect of anti-adhesion molecules in animal models, recent failure of a murine anti-ICAM antibody in human stroke illustrates the difficulties in extrapolating therapeutic effects observed in the laboratory to clinical benefits in patients (Cheng et al. 2004).
References


