Role of Inflammation in Cerebral Ischemia-reperfusion Injury

The ischemic cascade usually goes on for hours but can last for days, even after restoration of blood circulation. Although reperfusion of ischemic brain tissue is critical for restoring normal function, it can paradoxically result in secondary damage, called ischemia-reperfusion injury. The definitive mechanisms involved in ischemia-reperfusion injury still remains obscure. However, recent studies suggest that inflammatory cells release the reactive oxygen species in ischemia-reperfusion injured tissue which play a critical role in tissue apoptosis. The up regulation of reactive oxygen species triggers the expression of chemokines, cytokines, adhesive molecules and infiltration of leukocytes. Furthermore, the complementary system cascade is activated and play a major role in ischemia-reperfusion injury of cells. In addition to direct cell damage, regional brain ischemia-reperfusion induces an inflammatory response involving complement activation and generation of active fragments such as C3a and C5a anaphylatoxins (Arumugam et al. 2009).

3.1 Role of Cytokines

Cytokines are mediators for regulating the innate and adaptive immune systems. They contain a group of small glycoproteins that are produced in response to an antigen. Brain has upregulation of cytokines in ischemia-reperfusion injury (Pan et al. 2007). In the injured brain, cytokines are expressed not only in the cells of the immune system, but also produced by the resident brain cells including neurons and glia (Kettenmann et al. 2011). In addition, it has been shown that peripherally derived cytokines are involved in brain inflammation. Thus, peripherally derived mononuclear phagocytes, T lymphocytes, NK cells and polymorphonuclear leukocytes produce and secrete cytokines and might
contribute to inflammation of the CNS (Kettenmann et al. 2011). These actions were numerous but they are primary mediators for pro-inflammatory (TNF-α, interleukins) and growth factors. In cerebral ischemia-reperfusion the most studied cytokines related to inflammation are tumor necrosis factor-α (TNF-α), the interleukins, IL-1β, IL-6, IL-20, IL-10 and transforming growth factor TGF-β (Li et al. 2007; Liesz et al. 2009). Cerebral infarction was exacerbated by IL-1β and TNF-α and diminished by TGF-β and IL-10 (Li et al. 2007; Liesz et al. 2009). TGF-β and IL-10 may be cerebroprotective in action. Lower levels of the anti-inflammatory IL-10 and increased production of pro-inflammatory cytokines appear in ischemia-reperfusion tissue. In I/R, high circulating IL-1β elevates circulating IL-6, which is otherwise known as cytokine up regulated. Moreover, the serum level of IL-6 correlates with brain infarct volume and is a powerful predictor of early neurological deterioration (Lakhan et al. 2009). This suggests that IL-6 does not have a direct influence on acute ischemic injury. IL-1β modulates p38 MAPK and the NF-κB pathway thereby induction of IL-20 (Hu et al. 2009).

3.2 Role of Chemokines

Chemokines (monocyte chemoattractant protein-1) are a class of cytokines that direct the immigration of inflammatory cells of blood, such as neutrophils and macrophages, towards the source of the chemokines. Therefore they play prominent roles in inflammatory cell recruitment and cellular communication. In ischemia-reperfusion injury, expression of chemokines occurs such as MCP-1 (monocyte chemoattractant protein-1), macrophage inflammatory protein-1α (MIP-1α) and fractakline. Thereby leukocyte infiltration was increased and cause deleterious effects (Lakhan et al. 2009).
3.3 Role of Neutrophils

Activated neutrophils are involved in cerebral damage induced by ischemia (Shimakura 2000). Furthermore, reperfusion can exaggerate the infiltration of circulating inflammatory cells, including neutrophils, T cells, monocyte/macrophages and other cells in the ischemic brain region. Neutrophils release cytokines and chemokines, especially excessive production of ROS and induction/activation of MMP (mainly MMP-9), which amplify the brain-inflammatory responses further by causing more extensive activation of resident cells and infiltration of leukocytes, eventually leading to disruption of the BBB, brain edema, neuronal death and hemorrhagic transformation (Jin et al. 2010).
3.4 Role of Cellular adhesion molecules

There is an increasing evidence suggesting that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of cerebral ischemia-reperfusion (Frijns et al. 2002). Cytokines are responsible for upregulation of CAM and migration of leucocytes (Frijns et al. 2002). Leucocytes are adhering to the endothelial surfaces and then moving on to the endothelial surfaces. The interaction between vascular endothelial cells and leucocytes was maintained by CAM. Three main groups of CAMs are the integrins, selectins, and the immunoglobulin gene superfamily. In the early stages of ischemia procurement and rolling up of regulatory leucocytes and selectins appear. CAMs are upregulated in the first days after stroke by various cytokines. They are responsible for the adhesion and migration of the leukocytes. Furthermore, leukocytes roll on to the endothelial surface and then adhere to the endothelial cells. Selectins, especially E- and P-selectins are upregulated and mediate leukocyte rolling and recruitment during the early stages of ischemia. Among the immunoglobulin gene superfamily, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesions molecule-1 (ICAM-1) have been extensively documented in cerebral ischemia-reperfusion injury tissues. ICAM-1 expression increases upon stimulation by cytokines in cerebral ischemia-reperfusion within hours. A number of animal studies have been shown that after transient or permanent focal ischemia-reperfusion, the upregulation of CAMs, especially P- and E-selectin, ICAM-1, preceded the invasion of neutrophils into brain (Ceulemans et al. 2010; Frijns et al. 2002; Cornejo et al. 1997).
3.5 Role of Matrix metalloproteinases (MMPs)

MMPs are a large family of extracellular proteases. They are responsible for degrading of all extracellular matrix and extracellular matrix constituents. They are proficient to digest various soluble protein factors and cell surfaces including adhesion molecules, receptors, most notably cytokines, chemokines and growth factors. In normal adult brain, expression of MMPs is very low to undetectable, but in injured brain tissues by reperfusion found many MMPs are up regulated (Romanic 1998). After brain injury, the expression of MMPs is observed in neurons, astrocytes, microglia and endothelial cells. Cerebral ischemia-reperfusion injury is associated with disruptive blood brain barrier (BBB with a biphasic disruption) leading to vasogenic edema and haemorrhage (Ceulemans et al. 2010; Romanic et al. 1998). The experimental studies have been demonstrated that the BBB breakdown and haemorrhage results, to the over expression and activation of MMPs (Romanic et al. 1998). In cerebral ischemia, mainly expressions of MMP-2 and MMP-9 have been implicated to worsen the condition. Specially, in the serum and brain tissues of acute ischemic stroke patients or animal models, elevated levels of MMP-9 were found (Cojocaru et al. 2012; Park et al. 2009).

3.6 Role of Regulatory T lymphocytes

Recent evidences showing that, after stroke, if distraction of the adaptive immune system and innate immune system occurs, it leads to development of life threatening infections. After stroke, immunomodulatory addition may be useful for preventing autoimmune reactions against CNS. Recently, regulatory T lymphocytes (T_{reg}) were shown to play a prominent role in protecting cells in a mouse model for stroke. Thymus-derived CD4^{+}CD25^{+}Foxp3 T_{reg} cells play an important part in controlling immune responses under physiological conditions
and in various systemic and CNS inflammatory diseases. T\textsubscript{reg} are generated by antigen-presenting or dendritic cells expressing the immunosuppressive mediator indoleamine 2,3-dioxygenase (IDO), the first enzyme in the kynurenine pathway, that degrades and converts tryptophan to kynurenine interferon-\(\gamma\) and TNF-\(\alpha\) which are both present at high levels in the ischemic brain, possibly in microglia. Depletion of T\textsubscript{reg} cells profoundly cause delayed brain damage and deteriorated functional outcome. Absence of T\textsubscript{reg} cells augmented post ischemic activation of resident and invading inflammatory cells including microglia and T cells, the main sources of cerebral TNF-\(\alpha\) and IFN-\(\gamma\), respectively. T\textsubscript{reg} cells prevent secondary infarct growth by counteracting excessive production of pro-inflammatory cytokines and by modulating invasion and/or activation of lymphocytes and microglia in the ischemic brain. Liesz et al. 2009 demonstrated that the T\textsubscript{reg} cells antagonize TNF-\(\alpha\) and IFN-\(\gamma\) enhanced production, which can implement inflammatory reactions were delayed in brain injury and that T\textsubscript{reg} cells were involved to secretion of IL-10, it is the important mediator of the cerebroprotective effect via suppression of pro-inflammatory cytokine production. IL-10 potently reduced infarct size in normal mice and prevented delayed lesion growth after T\textsubscript{reg} cells depletion (Lakhan et al. 2009).

### 3.7 Role of Complementary activating system

Complement system is now recognized as playing an important role in the pathogenesis of cerebral reperfusion conditions. Activation of complementary system cascade either by the classical, alternative or lectin pathways, results in the formation of a myriad of pro-inflammatory mediators that serve to tissue injury. Two early products of complement activation, the anaphylatoxins C3a and C5a, bind to their respective G protein coupled receptors leading to activation of downstream signaling pathways including the p38a MAPK pathway as well as PI3-K. Terminal complement proteins C5b-9 form a transmembrane pore known
as the membrane attack complex (MAC), when it is present in high numbers, may lead to cell death (Newton et al. 2012). In cerebral infarcted tissues higher levels of MAC was observed (Hua et al. 2000). However, the MAC has the capacity to induce a number of cellular signal transduction pathways (i.e., PKC, p38a MAPK) and the transcription factor NF-kB. An end-result of the activation of these cellular signaling pathways is the increased expression of both chemokines (IL-8, MCP-1, and RANTES) and cytokines (IL-1β, TNF-α) from cells in the region of injury (Risnes et al. 2003). In addition, these complement products also have been demonstrated to increase the expression of the cell adhesion molecules P- and E-selectin and ICAM-1 (Skeie et al. 2010). Thus, the ability to up regulate a number of inflammatory mediators suggests that complement activation likely plays a pivotal role in amplifying the inflammatory response as well as directly inducing tissue injury (Lakhan et al. 2009).
References


Role of Anti-oxidants in Cerebral Ischemia-reperfusion Injury

Antioxidants are exogenous (natural or synthetic) or endogenous compounds acting by various pathways including inhibiting ROS formation, scavenging reactive oxygen species or their precursors and binding to metal ions needed for catalysis of ROS generation. The natural anti-oxidant system can be classified into two major groups: enzymes and low molecular weight anti-oxidants. The enzymes include SOD, catalase, glutathione peroxidases, thioredoxines and other antioxidant enzymes. The low molecular weight anti-oxidants may be classified into directly acting anti-oxidants (savers or chain breaking anti-oxidants) and indirectly acting anti-oxidants (chelating agents). ROS scavengers are important in defense against oxidative stress. The dietary sources of exogenous anti-oxidants are carotenoids, vitamin C, vitamin K and alpha-lipoic acid and phytochemical sources of anti-oxidants are bioflavonoids (Rahman 2007).

4.1 Cerebroprotective role of exogeneous anti oxidants

4.1.1 Carotenoids

Carotenoids are precursor of Vitamin A which are lipid soluble anti-oxidants. β- Carotene is the important carotenoid which posses anti-oxidant activity somewhat analogous to that of vitamin E (Gilgun-Sherki et al. 2002). High intake of β-carotene was associated with decreased cerebral infarction risk (Gilgun-Sherki et al. 2002; Mitchell et al. 1999; Yamagata et al. 2013; Sato et al. 2008).
4.1.2 Vitamin E

Vitamin E consists of a group of fat-soluble tocopherols (α, β, δ, si, γ) and tocotrienols. It is a fat soluble vitamin known to be a potent antioxidant. Vitamin E breaks the free radicals chain reaction in cellular lipid membranes and scavenges the peroxyl and alcoxyl radicals. Vitamin E deficiency also influences the activities of SOD, catalase and glutathione peroxidase (Gilgun-Sherki et al. 2002; Gumustas et al. 2007). Recent studies showed that vitamin E alter SOD, catalase, glutathione-S-transferase in rats following cerebral ischemia and reperfusion (Gumuştas et al. 2007). And also, recent finding shows that tocopherol analogues have potent cerebroprotective action by reducing infarction after MCA occlusion in rats (Iwashita et al. 2003).

4.1.3 Vitamin C

Vitamin C or ascorbic acid is a water soluble vitamin. It is derived from dietary source and is an exogenous molecule. It acts as scavenger of free radicals and inhibits peroxidation of membrane phospholipids. Ascorbic acid levels of brain are 10 fold higher than plasma levels, thus indicating, ascorbic acid may have cerebroprotection potential. Recent studies showed that ascorbic acid has cerebroprotection against oxidative stress and neuronal damage after ischemia and reperfusion in diabetic rats (Iwata et al. 2010; Liu et al. 2009; Miura et al. 2009, 2006; Kim et al. 2008).

4.1.4 Alpha lipoic acid

Alpha lipoic acid is absorbed from diet and is a sulphur containing compound, similar to GSH, GSSG, S-methyl-glutathione and dihydrolipoic acid. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA) are potent antioxidants. These compounds efficiently scavenge the singlet oxygen, nitric oxide
and hydroxide radicals. They also chelate transitional metals such as Cu, Mn and Zn. These actions contribute in reducing oxidative stress and recycle other antioxidants. They raise the intracellular levels of glutathione and modulate the transaction factors activities especially that of NF-kB. Some researchers showed that alpha lipoic acid has cerebroprotection by reducing oxidative stress in ischemia-reperfusion injury in rats (Mitsui et al. 1999; Panigrahi et al. 1996; Xie et al. 2012; Wolz and Krieglstein 1996; Connell et al. 2011).

4.1.5 Flavonoids

Flavonoids obtain from natural sources with variable phenolic structures and are found in vegetables, grains, fruits, flowers, barks, roots, stems, tea and wine. Putative biological actions of flavonoids have beneficial health effects. Recently considerable interest has been focused on flavonoids, because of their anti oxidant, anti inflammatory and anti proliferative activities (Diaz et al. 2012; Zhang et al. 2011). Over production of reactive oxygen species during ischemia and reperfusion could cause imbalances in oxidative and anti-oxidative processes. Furthermore, these alterations worsen the condition by the involvement of inflammatory reactions. Reactive oxygen species and inflammatory reactions both exaggerate tissue damage leads tissue apoptosis. There is increasing evidence supporting the hypothesis that bioflavonoids can provide protection against neurodegenerative changes associated with cerebral ischemia (Challa et al. 2011; Neelima et al. 2009; Jones et al. 2000; Cho et al.2006; Scheff et al.2013).
References


