Chapter-II

STROKE

death diagnosis blockage
sudden types recovery
drugs therapy
treatment effects

Review of Literature
Stroke is the leading cause of death and disability in adults poses serious public health problems globally after cardiovascular disease and cancer, and its incidence is expected to rise day by day with the projected increase in the number of the aging population (Chauveau et al., 2010; Flynn, 2008). According to the World Health Organisation, over 20 million people a year, equating to one in every 400 people, suffer a stroke worldwide and of these 5 million will not survive. About one-sixth of all human beings suffer at least one stroke during their lifetime (Seshadri et al., 2006). This estimate breaks downs to one person having a stroke every 40 seconds and one person dying from a stroke every 3 minutes (Rosamond et al., 2008).

It is estimated that about one third of stroke victims die; another one third are disabled permanently (Wolf et al., 1991). Two-thirds of these fatal subjects occurred in the people living in the developed countries (W.H.O, 2002). The loss of these patients from the work force and the extended hospitalization they require during recovery make the economic impact of the disease one of the most devastating in medicine. In India, nearly 1,000 people succumb to stroke each day, while around 1,500 stroke survivors remain vocationally impaired and need help in their daily life (WHO).

In India it has presently turned into a major public hazard with approximately 2 million patients per year, and the incidences of stroke is likely to increase in the coming year due to increase in population, life expectancy, stress level and changing life style involving smoking, excess alcohol use. The last available estimates from Indian Council of Medical Research (ICMR), Government of India indicate that in 2004 there were 930,985 cases of stroke in India with 639,455 deaths and 6.4 million disability adjusted life years (Daily) lost.

**Type of stroke**

Stroke occurs when brain stops receiving oxygen and nutrients and this happens after the occlusion of blood capillaries carrying these to the brain are blocked. The effects of a stroke are determined by the extent and site of brain injury, but the clinical symptoms of stroke do not accurately predict its underlying cause or causes. Death rates for the different types of stroke are illustrated in the Table 1.

**Table 1: Death rate (percentage) 30 days, 1 year, and 5 years after different types of stroke**

<table>
<thead>
<tr>
<th>Type of Stroke</th>
<th>30 days</th>
<th>1 year</th>
<th>5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic Stroke</td>
<td>10</td>
<td>23</td>
<td>52</td>
</tr>
<tr>
<td>Intracerebral haemorrhage</td>
<td>52</td>
<td>62</td>
<td>70</td>
</tr>
<tr>
<td>Subarachnoid haemorrhage</td>
<td>45</td>
<td>48</td>
<td>52</td>
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The two main types of stroke are ischemic and hemorrhagic (Fig 1.1), accounting for approximately 85% and 15%, respectively. Hemorrhagic stroke occurs when a blood leaks from the cardiovascular system resulting in reduced blood flow. Ischemic stroke accounts for approximately 85% of all stroke types and is characterized by restricted blood flow due to a blood vessel obstruction or an inability for the cardiovascular system to maintain adequate supply such as cardiac arrest (Lloyd-Jones et al., 2009). Hemorrhagic stroke has an intracerebral origin in 10% and a subarachnoid origin in 5% of all stroke cases (Lloyd-Jones et al., 2009).

![Fig. 1.1 Hemorrhagic stroke vs. Ischemic Stroke](image)

**Classification of cerebral ischemia**

Cerebral ischemia can be grouped into two major categories:

**A. Global or forebrain ischemia**

**B. Focal ischemia**

**A.** Global or forebrain ischemia occurs when cerebral blood flow (CBF) is reduced throughout the forebrain, is severe and of short or intermediate duration, followed by recirculation and a long recovery process (Siesjo et al., 1995). This form of ischemia is known to mimic changes in cerebral function which follow coronary artery bypass or heart attack in humans. In global ischemia of short duration followed by adequate reperfusion, the insult can be differentiated into three consecutive stages: initial events, free interval, and secondary cell death phase (Siesjo, 1993).

**B.** Focal ischemia is represented by a reduction in blood flow to a very specific brain region, (Wauquier et al., 1987) that leads to stroke, and it is usually the result of occlusion of a major
cerebral artery, such as the middle cerebral artery (MCA) (Siesjo, 1992). In this type of ischemia it is easy to discriminate between the core tissue, which is the central ischemic zone with relatively dense ischemia, and the penumbra tissues, located in more peripheral zones which are less dense due to their collateral blood supply from other major arteries (Siesjo et al., 1995). Focal ischemia can either be due to ischemic stroke or haemorrhagic in origin (Fig. 1.2).

![Focal ischemia diagram]

**Fig. 1.2 Classification of Focal ischemia**

**a. Ischemic stroke**

Ischemic stroke is defined as a situation of severely or completely blocked blood flow to an area of the brain, leading to neuronal dysfunction and cell death of the brain tissue in that area. There are two reasons why this might happen.

1) Thrombosis
2) Embolus

**Thrombosis stroke**

In thrombotic stroke, a thrombus (blood clot) usually forms around atherosclerotic plaques. Since blockage of the artery is gradual, onset of symptomatic thrombotic strokes is slower. A thrombus itself (even if non-occluding) can lead to an embolic stroke if the thrombus breaks off, at which point it is called an "embolus". Thrombolic stroke can be divided into two types depending on the type of vessel the thrombus is formed: Small Vessel disease and Large vessel problem.

**Embolic stroke**

An embolic stroke refers to the blockage of an artery by an embolus, a travelling particle or debris in the arterial bloodstream originating from elsewhere. An embolus is most frequently a thrombus, but it can also be a number of other substances including fat (e.g. from bone
marrow in a broken bone), air, cancer cells or clumps of bacteria (usually from infectious endocarditis). Because an embolus arises from elsewhere, local therapy only solves the problem temporarily. Thus, the source of the embolus must be identified.

b. **Hemorrhagic stroke**

Hemorrhagic strokes result in tissue injury by causing compression of tissue from an expanding hematoma or hematomas. This can distort and injure tissue. In addition, the pressure may lead to a loss of blood supply to affected tissue with resulting infarction, and the blood released by the brain hemorrhage appears to have direct toxic effects on brain tissue and vasculature.

(Fig.1.3) Mainly two type of hemorrhagic stroke

1) Intracranial hemorrhage

2) Intracerebral hemorrhage (ICH)

**Intracranial hemorrhage** Intracranial hemorrhage is the accumulation of blood anywhere within the skull vault.

**Intra-axial hemorrhage (blood inside the brain)—** is due to intraparenchymal hemorrhage or intraventricular hemorrhage (blood in the ventricular system).

**Extrablood inside the skull but axial hemorrhage (outside the brain)—** are epidural hematoma (bleeding between the dura mater and the skull), subdural hematoma (in the subdural space) and subarachnoid hemorrhage (between the arachnoid mater and pia mater).

**Intracerebral hemorrhage (ICH)**

Bleeding directly into the brain tissue, forming a gradually enlarging hematoma (pooling of blood). It generally occurs in small arteries or arterioles and is commonly due to hypertension, trauma, bleeding disorders, amyloid angiopathy, illicit drug use (e.g. amphetamines or cocaine), and vascular malformations. The hematoma enlarges until pressure from surrounding tissue limits its growth, or until it decompresses by emptying into the ventricular system, CSF or the pial surface. A third of intracerebral bleed is into the brain's ventricles. ICH has a mortality rate of 44 percent after 30 days, higher than ischemic stroke or even the very deadly subarachnoid haemorrhage.

**ARTERIES TO THE BRAIN**

The brain makes up only 2% of the total body weight, but when the body is at rest, it receives 20% of the cardiac output and consumes 3-4 ml O₂/min/100 g tissue, and this can represent up to 20% of all inhaled oxygen. When collateral cerebral circulation is altered, oxygen consumption in the ischemic zone is dramatically reduced, thus inducing significant neuronal injury (Kaushal and Schlichter, 2008; Sims and Zaidan, 1995). The anterior two-thirds of the brain are supplied by a pair of internal carotid arteries and the posterior third of the brain by a
pair of vertebral arteries (Fig. 1.3). These four arteries anastomose at the base of the brain to form the circle of Willis that provides excellent circulation to the brain.

![Diagram of the brain](image)

**Fig. 1.3 The area of the brain supplied by the cerebral arteries**

There are two main arterial systems by which brain cells need a constant supply for their survival and healthy functioning:

A) **Carotid artery**

In human anatomy, constant flows of blood to the brain are maintained by a pair of carotid arteries that supplies the head and neck. Each common carotid divides into an external carotid artery, which supplies blood to the face and an internal carotid artery which supplies the blood to the brain. The right carotid artery branches from the brachiocephalic artery and extends up to the right side of the neck. The left common carotid artery branches from the aorta and extends up the left side of the neck. The carotid arteries supply oxygenated and nutrient filled blood to the head and neck regions of the body.

B) **Basilar artery**

In human anatomy, the basilar artery is one of the arteries that supply the brain with oxygen-rich blood. The two vertebral arteries and the basilar artery are sometimes together called the vertebrobasilar system, which supplies blood to the posterior part of circle of Willis and anastomoses with blood supplied to the anterior part of the circle of Willis from the carotid arteries (Fig. 1.4).
Pathophysiology of Stroke

Cerebral ischemia results from decreased or interrupted blood supply leading to insufficient cerebral blood flow (CBF), thereby causing cellular energy crisis that initiate a complex series of physiopathological mechanism, eventually leading to irreversible cell damage. The mechanisms of cell death during brain ischemic insult are thought to largely arise from glutamate excitotoxicity, ionic imbalance, oxidative/nitrosative stress and activation of apoptotic pathway. However, emerging evidence also implicates other detrimental processes including inflammation, tissue acidosis and peri-infarct depolarization, as well as dysfunctions of endoplasmic reticulum and mitochondrion. (Mergenthaler et al., 2004)

Necrotic cell death in the central nervous system follows acute ischemia. It occurs in areas that are most severely affected by abrupt biochemical collapse, which leads to the generation of free radicals and excitotoxins (e.g., glutamate, cytotoxic cytokines, and calcium). The histologic features of necrotic cell death are mitochondrial and nuclear swelling, dissolution of organelles, and condensation of chromatin around the nucleus. These events are followed by the rupture of nuclear and cytoplasmic membranes and the degradation of DNA by random enzymatic cuts in the molecule. Given these mechanisms and the rapidity with which the process occurs, necrotic cell death is extremely difficult to treat or prevent.

Cerebrovascular disorder (CVD) is the major cause of morbidity and mortality worldwide and is predominantly caused by atherosclerosis (Lammeren 2011). Atherosclerosis is an inflammatory process resulting in local plaque deposition in the vessel wall of arteries, leading to luminal
narrowing and decreased tissue perfusion. Ischemic stroke may arise from the atherosclerotic large cerebral arteries (e.g., carotid, middle cerebral and basilar arteries) or atherosclerotic small cerebral arteries (e.g., lenticulostriate, basilar penetrating, and medullary arteries). In brain the stroke generally is a characteristic of large artery blockage rather than small arteries supplying deep cerebral white matter. Atherogenesis is a decades-long process in which the lumen of a blood vessel becomes narrowed by cellular and extracellular substances to the point of obstruction (Breslow, 1996, Pruissen et al., 2009, Fig. 1.5). It was seen that the earliest lesion of atherosclerosis is the fatty streak (Stary, 1994). On microscopy, the lesions primarily consist of lipid-filled macrophages (foam cells).

**Arterial wall injury**

Atherosclerosis is a degenerative vessel disorder that frequently affects large- to medium-sized arteries. Intima thickening and accumulation of lipid-loaded cells underlying the endothelium of large arteries, namely, fatty streaks or dots, is a hallmark of early-stage atherosclerotic lesions. These lipid loaded cells mainly originate from blood-born monocytes subsequently differentiate into macrophages. These macrophages engulf a large amounts of lipids deposited in the subintima space and take on the appearance of foamy structure, designated as foam cells (Watanabe T, 1989, Fig.1.6), which is accompanied by further destruction of the vessel wall and the accumulation of T-lymphocytes and macrophages (Hansson, 1989, Stary, 2000).

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**Fig. 1.5 shows narrowing of artery by atherosclerosis**

**Fig. 1.6 The major cellular events in the progression of atherosclerosis**
In later stages, atherosclerosis plaques exhibit a necrotic core, cholesterol clefts and calcifications. Atherosclerosis plaque rupture is associated with (1) inflammation, including the secretion of cytokines [e.g. interleukin (IL)-1α, IL-12, IL-18], (2) collagen-degrading. Pathophysiologic classification of vascular injury divided into three types (Fig.1.7).

1) A chronic minimal injury characterized by functional alterations of endothelial cells without significant morphologic changes (i.e., epithelial denudation), is thought to be caused primarily by the turbulence of blood flow.

2) Injury is characterized by denuding of the endothelium and superficial intimal injury. i.e. injury of soft plaques, with thrombus incorporation, is thought to be a major mechanism for the progression of atherosclerosis.

3) Injury is typified by deep intimal and medial damage, accompanied by marked platelet aggregation and mural thrombosis.

Role of Monocytes

Monocytes are the primary inflammatory cell type that infiltrates early atherosclerotic plaques. Oxidised LDL promotes endothelial cell damage and increases the expression of adhesion molecules on endothelial cell surfaces allowing circulating monocytes to attach. Adhesion of circulating monocytes (white blood cells that become particle-ingesting macrophages once they enter another tissue) to the surface of intact endothelial cells appears to be an early event in the development of atherosclerotic lesions monocyte binding to the endothelium of animals fed a high-cholesterol diet is preceded by expression of the vascular cell adhesion molecule (VCAM) and that is a lipid, ultimately responsible for activating the gene for VCAM (Gimbrone et al., 1995) Immune mechanisms also appear to play a role in atherogenesis (Ip et al, 1990, Pasceri et al., 2000), lymphocytes (cells responsible for the cell-mediated immune response) have been found to be present, albeit in small numbers, in both early fatty lesions and in advanced fibrous lesions in humans. As atherogenesis progresses, these macrophages take on a "foamy" appearance thus their designation as "foam cells" (Fig.1.8) and become one of the primary components of the fatty streak. In the late showed that the hepatic receptor for the low-density-lipoprotein (LDL) particle removes cholesterol from the bloodstream; however, this receptor was not found to
play a role in the accumulation of lipids within the foam cells of atherosclerotic lesions. Subsequently, the two investigators demonstrated that another type of LDL receptor, called the "scavenger receptor," was present on macrophages (Brown et al., 1986). Later work by Steinberg (1989) and colleagues showed that whereas normal LDL is not readily taken up by the scavenger receptor, this receptor does recognize oxidized LDL particles (Ballantyne et al., 2003). Oxidation of LDL cholesterol is induced by free radicals produced by macrophages, endothelial cells, or smooth-muscle cells.

**Role of Platelets**
Platelet aggregation and thrombosis may be promoted by toxic products released by macrophages and by moderate damage to the intimal surface with denudation of the platelets release growth factors that stimulate migration and proliferation of smooth muscle cells and also contribute to the formation of subendothelial "fibrointimal lesions" (Fig. 1.9) and possibly to formation of the outside capsule of predominantly "fatty lesions".

**Plaque fissuring and formation**
Plaques in the coronary arteries have undergone fissuring indicate that the majority are composed of eccentrically situated lipids (i.e., located in an area where the vessel bifurcates) that do not have an internal lattice of collagen supporting the cap of the plaque. The vulnerability of such a structure to fissuring appears to be related to circumferential stress on the plaque cap in systole, as well as infiltration of the cap tissue with foam cells (with reduction of total collagen content and a concomitant fall in tensile strength) (Rekhter et al., 1998). It is unclear whether foam cells weaken the tissue by passively distorting the spatial arrangement of the connective tissue matrix or by actively destroying connective tissue matrix protein by lytic mechanisms.

**Ischemic cascade leading to cell death**
**Energy failure causes onset of ischemia cascade**
The human brain is totally depends on oxygen and glucose to generate sufficient ATP by oxidative phosphorylation to maintain and restore ionic gradients. The brain’s energy metabolism is unique from other organs, since it requires a high metabolic rate, stores limited intrinsic energy. Energy failure is not the immediate cause of cell death, however, since all brain cells tolerate loss of ATP for several minutes. In humans, it appears that 5 to 10 minutes of complete occlusion is required for irreversible brain damage. In actuality, most strokes do not involve a complete occlusion of blood flow, but even a partial occlusion, if allowed to continue
for a sufficient time, may produce irreversible brain damage. Any energy interruption can quickly affect ion gradients (Hayashi and Abe, 2004). It is likely that the key process which triggers ischemic brain damage is the failure of cerebral energy metabolism.

The lack of oxygen and glucose supplies to the brain contributes to the failures of the synthesis of ATP (Siesjo, 1992). Under physiological conditions, normal brain energy metabolism, through glycolysis, tricarboxylic acid (TCA) cycle and oxidative phosphorylation, yields a net 36 adenosine triphosphate (ATP) per glucose molecule. The energy produced is used to maintain cellular homeostasis and basic neuro-function, for example the sodium-potassium ATPase (Na+–K+ ATPase) (Yang et al., 1992). Na+–K+ ATPase, an active pump that consumes neuronal ATP, is seriously affected by ATP depletion, resulting in a reduction of activity and membrane depolarization. One estimate suggests that the Na+ /K+ ATPase found on the plasma membrane of neurons, consumes 70% of the energy supplied to the brain (Robin et al., 2011). This ion pump maintains the high intracellular K+ concentration and the low intracellular Na+ concentration necessary for the propagation of action potentials. After cerebral ischemia, mitochondrial inhibition of ATP synthesis leads to ATP being consumed within two minutes, this causes neuronal plasma membrane depolarization, release of potassium into the extracellular space and entry of sodium into cells (Caplan 2000). Energy failure also prevents the plasma membrane Ca2+ ATPase from maintaining the very low concentrations of calcium that are normally present within each cell. The failure to synthesize adequate amount of ATP is usually accompanied by a rise in cytosolic calcium concentrations, since the energy-dependent efflux via ATP driven transporter and 3Na+/Ca2+ exchange can no longer control the influx of calcium. Moreover, the failure of calcium reuptake by the endoplasmic reticulum (ER) is a major consequence of ATP depletion (Hansen, 1985; Siesjo, 1993). Energy failure leads to several processes that threaten cell survival. Anaerobic glycolysis are utilized in the affected region to compensate for the loss of oxygen and provide a source of energy. However, this produces damaging by products, including lactic acid and hydrogen ions, which accumulate in tissue in proportion to the carbohydrate stores present at the outset of ischemia. Toxicity of hydrogen ions, especially their ability to facilitate ferrous-iron-mediated free-radical mechanisms, appears to irreversibly affect neuronal integrity.

When anaerobic glycolysis is stimulated, lactic acid production increases and results in intra- and extracellular acidosis (Macdonald and Stoodley, 1998). The depolarization activates voltage dependent calcium channels and releases excitatory amino acids into the extracellular space (Stys, 2005). A massive release of excitatory amino acid activates the glutamate receptors, leading to membrane depolarization and accumulation of free cytosolic calcium by cellular influx at the postsynaptic site (Meldrum BS 2000, Gupta and Briyal, 2004). The accumulation of
calcium plays a key role in the propagation of the irreversible neuronal damage by activation of
series of neurotoxic events such as lipid peroxidation, free radical generation, activation of
proteolytic enzymes and pathological gene activation leading to the formation of zone of
infarction in the area where blood supply has been interrupted.

**Disruption of ion homeostasis**

Ionic imbalance plays a critical role in pathogenesis of ischemic cell damage. Ischemia-induced
perturbation of ion homeostasis leads to intracellular accumulation of Ca^{2+} and Na^{+} which
results in the subsequent activation of numerous events including mitochondrial dysfunction
and eventual cell death. Dysregulation of mitochondrial Ca^{2+} plays a critical role in cell damage
under pathological conditions including traumatic brain injury and stroke (Liu et al., 2009). Many
lines of evidences suggest that Ca^{2+} is a key regulator of mitochondrial function. Mitochondrial
Ca^{2+} overload triggers the mitochondrial death pathway, which features the loss
of mitochondrial membrane potential, the opening of the mitochondrial permeability transition
transition pore (PTP), release of Cytochrome c, and enhanced generation of reactive oxygen
species (Orrenius et al., 2003).

An excessive influx of calcium damages the mitochondria by further exacerbating energy
failure, resulting in mitochondrial depolarization and swelling (Smith, 2004). Experimental
animal studies show that ATP and adenosine diphosphate (ADP) decrease significantly in acute
ischemia followed by an increase in inorganic phosphate and lactate (Faden et al., 1990; Gusev
& Skvortsova, 2002). This low level of ATP is insufficient to maintain the function of active ion
pumps, such as the Na^{+}-K^{+}-ATPase. Na^{+}-K^{+}-ATPase maintains a high intracellular
concentration of potassium and low intracellular concentration of sodium (Robin et al., 2011);
as a result, Na^{+} is no longer excluded from the cell and intracellular Na^{+} concentration
progressively increases. Consequently, this impairment in energy supply disrupts the ion
homeostasis resulting in a loss of membrane potential and depolarization of neurons. The
depolarization activates voltage dependent calcium channels and releases excitatory amino acids
into the extracellular space (Stys, 2005). At the same time, reuptake of excitatory amino acids by
glutamate transports is impeded due to loss of energy, which further increases the accumulation
of glutamate, the main excitatory amino acid in the central nervous system (Izumi et al., 2006).
Extracellular glutamate can bind to both iontropic and metabotropic glutamate receptors.
Iontopic receptors, such as N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-
isoazolepropionic acid (AMPA), and kinate receptor subtypes, are activated when glutamate
bind and allow an influx of calcium and sodium into the cell. Thus, the NMDA receptor pore is
open and Ca^{2+} flows into the cell. Glutamate can also bind to metabotropic receptors which are
G-protein coupled. Activation of G-protein cascade triggers intracellular calcium release
through phospholipase C and phosphoinositol (Pellegrini-Giampietro, 2003) via internal Ca\textsuperscript{2+} release from endoplasmic reticulum reserves and mitochondria (Lehotsky et al., 2003). Elevation of intracellular calcium levels is a pivotal process leading to irreversible neuronal death by activating several harmful enzymes (Cheung et al., 1986; Kirino, 1986) such as proteases, phospholipases, protein kinases, protein phosphatases, guanylyl cyclases, and endonucleases (Nagahiro et al., 1998). In addition, the disrupted ion homeostasis changes the osmotic pressure; water enter the cell and causes edema in brain tissue (Fu et al, 2007). The cellular integrity begins to breakdown and solutes begin to leak into the cell causing further cell and tissue swelling. Since the intracranial cavity is a fixed rigid structure, tissue swelling further restricts cerebral blood flow, which causes a vicious cycle that promotes cerebral edema and ischemia (Panickar KS, Anderson, 2011).

**Excitotoxicity**

Excitotoxicity is considered to play a central mechanism underlying neuronal death in stroke (Rothman SM, 1986, Choi DW 1990). A substantial body of evidence has implicated excitotoxicity is considered to trigger tissue damage in both focal experimental ischemia (Siesjo, 1995, Arundine and Tymianski, 2004) and clinical ischemia. Glutamate is the principal excitatory neurotransmitter in the mammalian brain, which has an essential role in intracellular communication, plasticity, growth and differentiation (Mehta et al., 2007). This amino acid also mediates excitatory synaptic transmission via a number of specific receptors. Glutamate is released at high concentrations in the penumbral cortex (Takagi K, 1993), particularly if blood flow is reduced for a long period, and the amount of glutamate released correlates with early neurological deterioration in stroke patients. Intracellular calcium levels are known to predict exotoxic cell death by activation of the glutaminergic NMDA receptor (Hyrc et al., 1997). Excess activation of ionotropic glutamate receptors cause fulminant neuronal death, namely glutamate neurotoxicity or excitotoxicity, influx and accumulation of Ca\textsuperscript{2+} and Na\textsuperscript{+} that result in rapid swelling and subsequent neuronal death within a few hours. Impaired energy increases presynaptic glutamate release through membrane depolarization and the subsequent activation of the voltage gated Ca\textsuperscript{2+} channel. It also interferes with the re-uptake of glutamate (primarily into astrocytes), which results in the abnormal accumulation of synaptic glutamate.

The excitotoxicity can contribute to neuron death by altering the functions of mitochondria. Mitochondrial disturbance is the result of both oxidative-nitrosative stress and a direct effect of excessive Ca\textsuperscript{2+} intracellular levels. Major excitotoxic events promoted by cytoplasmic Ca\textsuperscript{2+} overload due to massively activated glutamate receptors include mitochondrial dysfunction, oxidative/nitrosative stress. Mitochondria play an important role in calcium homeostasis (Berridge, 2005; Nicholls, 2005). Under conditions of cytoplasmic excess of Ca\textsuperscript{2+}, mitochondria
are very important for cell survival, as they have the ability to sequester large amounts of Ca\(^{2+}\). The excitotoxic mechanisms which lead to neuron death are complex, but primarily involve the generation of free radicals (Pelligrini-Giampietro DE, 1990, Oh and Betz, 1991), mitochondrial dysfunction and the participation of various transcription factors as activators of gene expression (Clemens et al, 200). All of these mechanisms acting synergistically can damage cellular proteins (Berlett BS, 1997), lipids (Sakamoto A, 1991) and DNA (Hayashi T, 1999), which leads to the deterioration of cellular architecture and signalling, resulting necrosis, apoptosis or both depending on the severity of the insult and of relative speed of each process (Bonfoco et al,1995, Unal-Cevik et al, 2004).

**Blood brain barrier breakdown**

The blood-brain barrier (BBB) is the specialized system of capillary endothelial cells that protects the brain from harmful substances in the blood stream, while supplying the brain with the required nutrients for proper function. Unlike peripheral capillaries that allow relatively free exchange of substance across / between cells, the BBB strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers. Thus the BBB is often the rate limiting factor in determining permeation of therapeutic drugs into the brain (Fig. 1.10). Additionally, BBB breakdown is theorized to be a key component in stroke associated pathologies (Wang et al., 2011; Michalski et al., 2010).

**Brain edema**

Cerebral edema is characterized by accumulation of excessive fluid in the substance of the brain. The brain is especially susceptible to injury from edema, because it is located within a confined space and cannot expand. Brain edema is also known as brain swelling, and wet brain (Fig. 1.15). Brain edema can be classified into two different types on the basis of morphological characteristics:

1. Vasogenic or “wet” edema, the result of an increased BBB permeability, and
(2) Cytotoxic or “dry” edema, the result of the actual swelling of the cells of the brain parenchyma (Klatzo, 1967).

- Vasogenic edema is the type of edema most often present in the brain after injury, induced by ischemic stroke, brain tumors or inflammatory lesions. The BBB expresses morphological changes during the onset of vasogenic brain edema, such as the opening of tight junctions and a damaged endothelial cell membrane, followed by migration of leukocytes into the CNS (Klatzo, 1987).

- Cytotoxic brain edema is the most prominent clinical disorder after ischemic processes in the CNS and is characterized by an increase in the water content of the cells of the CNS, which may caused by a disturbance in the transport systems for potassium and sodium rather than from changes in the permeability of the BBB.

Mitochondrial dysfunction

Mitochondria play a central role in cellular metabolism and are responsible for cellular respiration that generates energy in the form of ATP through oxidative phosphorylation via the electron transport chain, which contains five multi-subunit enzyme complexes, I to V (Clarkson et al., 2007). The cell uses this energy to perform the specific work necessary for cell survival and function. Nerve cells in the brain require a great deal of energy, and thus appear to be particularly damaged when mitochondrial dysfunction occurs particularly in stroke. Mitochondria are centrally involved in the generation of free radicals, as they generate huge numbers of oxidative-reduction reactions and use massive amounts of oxygen. Reactive oxygen species (ROS) are generated in complex I and complex III during mitochondrial respiration (Claude et al., 1996). Much evidence suggests a major role for mitochondrial dysfunction in the pathogenesis of stroke, and in particular, defects in mitochondrial complex-I and complex-III of the respiratory chain. A complex-I defect could most obviously contribute to neuronal degeneration in stroke through decreased ATP synthesis as well as damage caused by excess production of ROS. Therefore, mitochondrial oxygen metabolism can be a potential threat to tissues and cells (Niizuma et al., 2010).

Mitochondrial release of multiple apoptogenic proteins has been identified in ischemic and post-ischemic brain, mostly in neurons. Changes in the permeability of the mitochondrial outer membrane result in a non-reversible step in cell death processes. Cytochrome c released from mitochondria binds in the cytoplasm to Apaf-1 to initiate the formation of an apoptosome along with ATP, which then binds pro-caspase-9 then, activates “executioner” caspases, which in turn proceed to cleave key substrates in the cell. Members of the Bcl-2 family are proapoptotic or antiapoptotic. Pro-apoptotic members of the Bcl-2 family (Bad), which bear resemblance to
channel-forming bacterial toxins, induce mitochondrial membrane permeabilization when added to purified mitochondria. The balance between pro-apoptotic and anti-apoptotic signals from the Bcl-2 family has a crucial role in the release of cytochrome c. Therefore, mitochondria have been identified as targets of cytoprotection for several disease states including stroke, mainly due to the high susceptibility of the organelle to cellular insults (Mehta and Li, 2009). In the brain, the mitochondria are especially vulnerable due to the high metabolic activity of the brain that is key in producing significant levels of cell-damaging oxidants (Szeto, 2006).

Oxidative stress

There has been a considerable body of evidence over the last few years to suggest that oxidative stress associated with excessive production of reactive oxygen species (ROS) / reactive nitrogen species (RNS) is a fundamental mechanism of brain damage in stroke and reperfusion ensuing stroke (Chan, 1996; Neumar, 2000; Khan et al., 2009). Oxidative stress culminates due to an imbalance between pro-oxidants and antioxidants and consequent excessive production of reactive oxygen species. Reactive oxygen species are biphasic, playing a role in normal physiological processes and are also implicated in a number of disease processes, whereby they mediate damage to cell structures, including lipids, membranes, proteins, and DNA. The cerebral vasculature is a major target of oxidative stress playing a critical role in the pathogenesis of ischaemic brain injury following a cerebrovascular attack (Ischemic stroke). The brain is a target for many reasons, including high concentrations of peroxidisable lipids, low levels of protective antioxidants, high oxygen consumption, and high levels of iron that act as pro-oxidants under pathological conditions, and reactions involving dopamine and glutamate oxidation also occur in the brain (Saeed et al., 2007). However, in certain physiological processes, ROS have been shown to be beneficial and play a role in cell signalling, the induction of mitogenic responses, immune defence, cellular senescence, apoptosis, and the breakdown of toxic compounds (Bergendi et al., 1999).

a) Free Radical Damage

The brain and nervous system are particularly vulnerable to free radical damage for a number of reasons. The membrane lipids in the brain contain high levels of polyunsaturated fatty acid side chains, which are prone to free radical attack, and are readily peroxidisable, contributing to structural and functional perturbations of the membrane and cell function. The brain also consumes large quantities of total oxygen for its relatively small weight, contributing further to the formation of reactive oxygen species. It has been estimated that up to 2% of the oxygen consumed by healthy mitochondria is converted to superoxide, and this amount is higher in damaged mitochondria.
b) Reactive oxygen species
Superoxide (O$_2^-$) is the primary ROS and generates H$_2$O$_2$ by dismutation. Superoxide is produced in tissues via a number of enzymatic reactions and common cellular sources of O$_2^-$ include auto-oxidation of small molecules, including haemoglobin and myoglobin, mitochondrial components and oxidative enzymes, e.g. nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, cyclooxygenases, and oxidation of unsaturated fatty acids (Stamler et al., 1992) (Fig. 1.1). The rate of O$_2^-$ production is dependent on the concentration of the oxidisable substrates, the availability of oxygen and the activity of antioxidant enzymes. Unlike H$_2$O$_2$ and the hydroxyl radical (’OH), O$_2^-$ is an oxidizing and reducing compound (Bergendi, 1999). ’OH is generated from H$_2$O$_2$ in the presence of ferrous iron that has been reduced by O$_2^-$ by a chemical process known as the Haber- Fentons reaction. H$_2$O$_2$ is formed by the dismutation of O$_2^-$ or direct reduction of oxygen, and is highly lipid soluble.

Fig. 1.11 Basic Mechanism of ROS mediated cell damage

Superoxide radicals (O$_2^-$') generated during oxidative metabolism, are neutralized to water via a two-step process involving superoxide dismutase (SOD) in the first step, and both or either glutathione peroxidase (GPx) and catalase in the second step. An imbalance in this pathway favors the build-up of hydrogen peroxide (H$_2$O$_2$). Fenton-type reactions occur when H$_2$O$_2$ interacts with transition metals such as Fe$_2^+$, resulting in the production of noxious hydroxyl radicals (’OH). These radicals initiate rounds of peroxidative damage to molecules such as lipids, via the production of lipid peroxy radicals (LOO$^-$) and lipid hydroperoxides (LOOH). The functional importance of GPx may reside in its ability to remove both hydrogen and lipid
peroxides and neutralize these to water and lipid alcohol (LOH), respectively. (J.B. de Haan et al.)

c) Reactive nitrogen species

Reactive nitrogen species (RNS) are also generated under normal physiological and pathological conditions. The nitric oxide radical (NO•) is generated in biological tissues by specific nitric oxide synthases (sNOS), and neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) that metabolise L-arginine to L-citrulline (Ridnour, 2004). Generation of NO• and O2- favors the production of a toxic reaction product, peroxynitrite anion (ONOO•) very powerful oxidant (Beckman & Koppenol, 1996). NO• regulates neural signalling, blood pressure, smooth muscle relaxation and immune surveillance. NO• is both aqueous and lipid soluble, readily diffusing through the cytoplasm and plasma membranes. During the inflammatory process, immune cells produce O2 and NO•, which react to produce the peroxynitrite anion (ONOO•), a potent oxidising agent that can cause DNA fragmentation (Sestili et al., 2000, Beckman, 1990) and lipid peroxidation (Radi et al., 1991; Rubbo et al., 1994).

Lipid peroxidation

Lipid peroxidation is one of the major outcomes of free radical-mediated injury that directly damages biological membranes and generates a number of secondary products that possess neurotoxic activity (Halliwell and Chirico, 1993). Lipid peroxidation has been defined as the oxidative deterioration of polyunsaturated lipids, i.e. those lipids containing more than two carbon-carbon double covalent bonds (Aruoma et al., 1991) (Fig. 1.12). Several experimental evidences indicate that extensive lipid peroxidation results in loss of membrane integrity, impairment of the function of membrane-transport proteins and ion channels, disruption of cellular ion homeostasis and eventual rupture leading to release of cell and organelle contents such as lysosomal hydrolytic enzymes (White et al., 2000). This process proceeds by free radical chain reaction mechanism.

Increased nitric oxide concentrations associated with ischemia may have dual effects on lipid peroxidation. Reaction of nitric oxide with superoxide causes the formation of peroxynitrite that initiates lipid peroxidation via reaction of lipids with its decomposition products hydroxyl

![Fig. 1.12 Basic Mechanism of ROS mediated Lipid peroxidation (LPO)]
radical and nitrogen dioxide (Brookes et al., 1998). In contrast, nitric oxide itself may directly inhibit lipid peroxidation by intercepting alkoxy and peroxyl radical intermediates thereby terminating chain propagation reactions (Nicolescu et al., 2002; Niziolek et al., 2003).

**Inflammation**

Inflammation has been implicated in the pathogenesis of experimental stroke models and the recruitment of inflammatory cells appears to worsen ischemic brain injury (Iadecola and Anrather, 2011). Many studies demonstrate that cerebral ischemia is associated with the infiltration of inflammatory cells to the ischemic region (Terao et al., 2008). It is histologically characterized by the infiltration of leukocytes, mainly polymorphonuclear leukocytes, monocytes/macrophages and astrocytes (Pantoni et al., 1998). Activation of these resident cells population along with immune cells stimulates the production and release of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 and the induction of iNOS and COX-2, and the activation of enzymes such as NADPH oxidase from the ischemic tissue (Carvalho-Tavares et al., 2000). In this inflammatory environment, cerebral endothelial cells increase their expression on cell surface adhesion molecules that mediate recruitment of leukocytes and platelets to the ischemic region (Stanimirovic, 1997).

Brain tissue is not well equipped with antioxidant defenses, so reactive oxygen species and other free radicals/oxidants, released by inflammatory cells, threaten tissue viability in the vicinity of the ischemic core (Lakhan et al., 2009). During ischemic injury, intracellular Ca^{2+}, free radicals, and hypoxia induce the expression of many pro-inflammatory genes and increase the synthesis of transcription factors such as nuclear factor-kB, interferon regulatory factor 1, and STAT3 for the regulation of inflammatory cytokine production (Salminen et al., 1995; Iadecola et al., 1999; Wen et al., 2006). The development of post-ischemic brain inflammation is co-ordinated by the activation, expression and secretion of numerous pro-inflammatory mediators from the brain parenchyma and vascular cells including cytokines, leukotriens and adhesion molecules (Giulian et al., 1993; del Zoppo, 2000). Pro-inflammatory cytokines, such as TNF-α and IL-1β (Salminen et al., 1995; Wang et al., 2009), and anti-inflammatory cytokines, such as IL-6 and IL-10 (Vila et al., 2003; Sotgiu et al., 2006) are released by the injured ischemic brain cells. In response to the ischemic injury the endothelial cells express adhesion molecules including intercellular adhesion molecule 1(ICAM-1), P-selectins, and Eselectins on their surface to attract neutrophils and macrophages (Haring et al., 1996). The hallmark of cerebral ischemia inflammation is neutrophil infiltration (Weston et al., 2007). Neutrophils are known to release injurious mediators (Tomita and Fukuuchi, 1996). They also contribute to ROS generation (\( \cdot O_2 \) & H\(_2\)O\(_2\)) via NADPH oxidase (Ellis et al., 1998).
Apoptosis

Apoptosis is a form of programmed cell death that occurs in neurons during development of the nervous system and may also be a prominent form of neuronal death in both acute and chronic neurodegenerative disorders such as stroke (Mattson et al., 2000). Cell death helps to regulate cell numbers. In the case of neuronal development, cell death adjusts the number of nerve cells to match the number of target cells that require innervation (Penaloza et al., 2008). The term apoptosis was first used by Kerr et al in 1972 and is Greek in origin, meaning “dropping off” of petals or leaves from plants or trees (Kerr et al., 2002). Apoptosis or “programmed cell death” is a process by which cells undergo physiological cell death in response to diverse stimuli and is essential for normal biological processes such as morphogenesis, tissue homeostasis, and the elimination of damaged or virally infected cells, and may play a role in various pathologic and toxicological process (Maslinska, 2003). Necrotic cell death occurs in core areas that are most severely affected by abrupt biochemical collapse, which leads to the generation of free radicals and excitotoxins (e.g., glutamate, cytotoxic cytokines, and calcium). Apoptosis occurs in penumbra areas that are not severely affected by the injury. The penumbra is the battle ground for stroke therapy as it has been recognized as an area at risk and can be salvaged with appropriate intervention (Yuan, 2009). Apoptosis differs from necrosis in the fact that apoptosis is an active process of cell destruction, characterized by intact plasma membranes, cell shrinkage and the formation of apoptotic bodies whereas necrotic cell death is often characterized by loss of membrane integrity, cell swelling and lysis (Henriquez et al., 2008, Fig. 1.13). Mitochondria are at the centre of apoptosis, also known as programmed cell death. Intra-cellular and extra-cellular signals alter the association of a set of cytosolic pro-apoptotic and anti-apoptotic proteins with the organelle. These include the Bax and Bcl-2 families of proteins and caspase family of cysteine proteases play critical roles in the activation, signal transduction and execution of apoptosis (Yuan, 2000; Hsu and Hsueh, 2000). In response to the oxidative load in mitochondria, the outer membrane of mitochondria becomes permeabilized (Endres et al., 1997) resulting in the translocation of Bax from the cytosol to the mitochondria and the release of cytochrome c, normally confined to the mitochondrial intermembrane space (Estaquier et al., 2012). The proapoptotic protein translocation is controlled by the family of Bcl-2 proteins (Tsujimoto, 2003). Release of cytochrome c into the cytosol leads to the formation of the apoptosome, a complex composed of apoptotic-protease activating factor-1, procaspase-9, and
ATP (Chan et al., 2001). The apoptosome permits the autoactivation of procaspase-9, which is followed by the activation of procaspase-3 (Chen et al., 1997). Active caspase-3 leads to cleave key cellular substrates that are required for normal cellular function including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. The caspases also activate other degradative enzymes such as DNases, which begin to cleave the DNA in the nucleus (Enari et al., 1998). The prominent molecular hallmark of apoptosis consists of nuclear fragmentation from chromatin condensation and internucleosomal DNA breakdown (Chan, 2001).

**ANTIOXIDANT SYSTEM IN THE BRAIN**

There are various antioxidant defense mechanisms present in the brain such as superoxide dismutase, catalase, glutathione peroxidase, and other reductants (glutathione, ascorbate, and alpha-tocopherol). Antioxidants are exogenous (natural or synthetic) or endogenous compounds that reduce generation of ROS by acting in several ways including removal of $^{1}O_2$, scavenging reactive oxygen species or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation.

Cellular antioxidant defence is classified into two categories: non-enzymatic and enzymatic (Table. II). Primary non-enzymatic antioxidant are vitamin C, vitamin E and ubiquinol.
etc, in addition thiol containing antioxidants such as reduced glutathione (GSH) which directly scavenge ROS (Fig. 1.14). Enzymatic antioxidants enzymes include superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), and catalase etc. Superoxide dismutase neutralizes superoxide anions into water (Chan, 2001). In superoxide dismutase deficient mice, neuronal death and edema in the brain increased (Kondo et al., 1997). Therefore, this shows that superoxide dismutase plays an important role in reducing the damage from oxidative stress. Glutathione peroxidase and catalase convert $H_2O_2$ into $H_2O$ (Chan, 2001). Catalase is more abundant in astrocytes than in neurons and present mainly in peroxisomes (Tang et al., 2007). Glutathione peroxidase uses glutathione to reduce $H_2O_2$ in water and is present in the cell cytosol. Glutathione, ascorbate (Vitamin C), and alpha-tocopherol (Vitamin E) also play important roles in protecting the brain from focal ischemia (Tang et al., 2007), which suggests that a person’s nutrition and the environment can affect the outcomes in cerebral ischemia.
Table II. Cellular Antioxidant Defence Systems

<table>
<thead>
<tr>
<th>Enzymes</th>
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<tbody>
<tr>
<td>Cu-Zn SOD (cytosol)</td>
<td>Mn SOD (mitochondria)</td>
</tr>
<tr>
<td>GSH peroxidase</td>
<td>GSH-S- transferase</td>
</tr>
<tr>
<td>GSSG reductase</td>
<td>Catalase</td>
</tr>
<tr>
<td>Quinone reductase</td>
<td></td>
</tr>
<tr>
<td>Repair System</td>
<td></td>
</tr>
<tr>
<td>Methionine sulphoxide reductase</td>
<td>DNA repair</td>
</tr>
<tr>
<td>Proteolysis of oxidised proteins</td>
<td>Phospholipase A2 acyl transferase</td>
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<tr>
<td>Ion sequestration</td>
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<tr>
<td>Transferrin</td>
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<tr>
<td>Ferritin</td>
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<tr>
<td>Lactoferrin</td>
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<tr>
<td>Ceruloplasmin</td>
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<tr>
<td>Metallothioneins</td>
<td></td>
</tr>
<tr>
<td>Small molecules</td>
<td></td>
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<tr>
<td>Ascorbate</td>
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<tr>
<td>GSH</td>
<td></td>
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<tr>
<td>Bilirubin</td>
<td></td>
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<tr>
<td>α-tocopherol</td>
<td></td>
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<tr>
<td>Ubiquinol</td>
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<tr>
<td>Urate</td>
<td></td>
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<tr>
<td>Carotenoids</td>
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</table>

Glutathione system

The glutathione system includes glutathione peroxidases, glutathione reductase, and glutathione S-transferase which is found in animals, plants and microorganisms (Creissen et al., 1996; Meister and Anderson, 1983). The tripeptide of glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine) is one of the most abundant intracellular thiol in cytosol, nuclei and mitochondria (Meister, 1981) representing the major soluble antioxidant in these cell compartments. In the brain, the concentration of GSH is ~ 2 mM (Rehncrona et al., 1980; Valdovinos-Flores and Gonsebatt, 2012) of which 0.3% is in the oxidized form (GSSG). GSH synthesis can be limited by the ATP availability (Shan et al., 1989; Hurtado et al., 2003). In nucleus, GSH maintains the redox state of critical protein sulphhydryls that are necessary for DNA repair and expression.

Oxidized glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism (Chi et al., 2005). GSH plays an important role in the protection of the cells from oxidative damage by reducing disulphide groups of proteins.
and other cellular molecules, or by scavenging free radicals and active oxygen species (McLennan et al., 1991). The product of the oxidation of GSH is glutathione disulfide (GSSG). GSH has a major intracellular antioxidant activity being involved in detoxification of peroxides and electrophilic toxins as a substrate for GSH peroxidase and GSH transferase. GSH reacts directly with radicals in non-enzymatic reactions being the electron donor in the reduction of peroxides catalyzed by selenium-containing glutathione peroxidase (GPx).

Glutathione peroxidase is the most abundant and is a very efficient scavenger of hydrogen peroxide, while it is most active with lipid hydroperoxides. Surprisingly, glutathione peroxidase is dispensable, as mice lacking this enzyme have normal lifespans, (Ho et al., 1997) but they are hypersensitive to induced oxidative stress (de Haan et al., 1998). GSH is regenerated from GSSG within the cells in a reaction catalyzed by GR (Fig. 1.1). This enzyme regenerates GSH by transferring reducing equivalent from NADPH to GSSG. NADPH regeneration during GSH redox cycling in brain depends on NADPH-regenerating enzymes such as glucose-6-phosphate dehydrogenase (G6PDH). G6PDH is a cytoplasmic enzyme that affects the production of reduced form of cytosolic nicotinoadenosine dinucleotide phosphate coenzyme (NADPH) by controlling the step from glucose-6-phosphate to 6-phosphogluconate in the pentose phosphate pathway (Beutler et al., 1996; Kletzien et al., 1994). This enzyme is highly conserved during evolution and plays multiple roles in the cell. Until recently, the role of this housekeeping enzyme in the cell response to the oxidative stress was limited to human erythrocytes that lack any other NADPH in producing route. However, recent observations have shown that the G6PDH also plays a protective role against reactive oxygen species in eucaryotic cells that possess alternative routes for the production of NADPH and that G6PDH expression is upregulated by oxidants through a mechanism acting mainly on the rate of transcription of this gene (Cramer et al., 1995; Salvemini et al., 1999). NADPH can also be regenerated by 6-phosphogluconate dehydrogenase (6PGDH) as well as malic enzyme (MEs), NADP+-dependent isocitrate dehydrogenases (ICDHs) and mitochondrial nicotinamide nucleotide transhydrogenase (Minich et al., 2003; Bukato et al., 1995). In addition, the glutathione S-transferases also show high activity with lipid peroxides (Sharma et al., 2004). These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism.

**Superoxide dismutase**

Superoxide dismutases catalyze the dismutation of superoxide into oxygen and hydrogen peroxide (Zelko et al., 2002; Bannister et al., 1987). SOD is present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005). Reactive oxygen metabolites have long been implicated in the development of brain lesions in reperfusion after cerebral ischemia. ROS
are usually scavenged by antioxidant enzymes, primarily by superoxide dismutase (Fig. 1.15). Superoxide is a key constituent in oxidative stress. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen (Warner et al., 2004). Based on the metal ion requirements and the anatomical distribution three major endogenous superoxide dismutases exist in mammalian brain cells: Copper-zinc superoxide dismutase (CuZn-SOD) (SOD1), magnese superoxide dismutase (Mn-SOD) (SOD2) and extracellular SOD (EC-SOD) (SOD3) (Chan, 1996). CuZn-SOD is found in the cytosol and nucleus of cells. CuZn-SOD containing copper and zinc at its active site. Mn-SOD containing manganese at its active site is located in the mitochondrial matrix. CuZn-SOD and Mn-SOD are highly expressed in neurons. The manganese superoxide dismutase (Mn-SOD) isoform located in mitochondria. The mitochondrial isozyme seems to be the most biologically important of these three, since mice lacking this enzyme die soon after birth (Melov et al., 1998). In contrast, the mice lacking copper/zinc SOD are viable but have lowered fertility, while mice without the extracellular SOD have minimal defects (Reaume et al., 1996). Cu, Zn-SOD and MnSOD are abundant in neural tissue. Cu, Zn-SOD over expression reduces ischemic damage resulting from ischemia/reperfusion (Yang et al., 1994), MnSOD targeted deletion worsens the outcome from both temporary and permanent middle cerebral artery occlusion (Kim et al., 2002; Murakami et al., 1998). The third SOD isoform (EC-SOD) is present in extracellular fluids such as plasma and is also expressed in the brain but at lower levels than Cu, ZnSOD and MnSOD (Marklund et al., 1982). In adult focal ischemia, EC-SOD over expression conferred protection (Fukui et al., 2002) and EC-SOD deficiency aggravated the injury.

![Superoxide dismutase mediated ROS scavenging mechanism](image)

Fig. 1.15 Superoxide dismutase mediated ROS scavenging mechanism
Catalase
Catalase is an antioxidant enzyme like superoxide dismutase (SOD) and glutathione peroxidase, is produced naturally within the body (Liddell et al., 2004). Catalase is some of the most efficient enzyme found in nearly all living organisms exposed to oxygen (del Rio et al., 1992); each catalase molecule can convert millions of hydrogen peroxide molecules every second. It catalyzes the decomposition of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani et al., 2004; Zamocky and Koller 1999) (Fig. 1.15). Catalase also uses hydrogen peroxide to break down potentially harmful toxins in the body, including alcohol, phenol, and formaldehyde. It also helps to prevent the conversion of hydrogen peroxide to hydroxyl radicals, potentially dangerous molecules that can attack on membrane, protein and damage DNA.

Nitric oxide synthase
Nitric oxide synthase (NOS), an enzyme that produces toxic amounts of nitric oxide, is expressed in a number of brain pathologies, including cerebral ischemia (Iadecola et al 1997). In cerebral ischemia it is produced by occlusion of the rat middle cerebral artery (MCA), NOS expression begins 12 h after induction of ischemia, peaks at 48 h, and subsides at 7 d (Iadecola et al., 1995). Nitric oxide (NO) is enzymatically synthesized from L-arginine requires a number of cofactors, namely, NADPH, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and tetrahydrobiopterin, as well as calmodulin. (Moncada and Higgs, 1995). Molecular oxygen is also used in this reaction, which proceeds via the synthesis of N-hydroxyarginine and results in the formation of citrulline in addition to NO. The three isoforms of mammalian NOS: endothelial NOS, neuronal NOS and inducible NOS, are products of distinct genes and share 50–60% homology (Alderton et al., 2001). Nitric oxide may also serve as an antioxidant against products of the Fenton reaction. At the same time, iNOS expression has been implicated as a critical factor for promoting posts ischemic neurogenesis (Zhu et al., 2003). Further, iNOS expression may contribute to increased tolerance of brain to ischemia induced by preconditioning stimuli (Kapinya et al., 2002) as does eNOS upregulation (Hashiguchi et al., 2004). The fact that eNOS and nNOS are Ca^{2+} and calmodulin-dependent and is present throughout the brain as well as in the spinal cord and peripheral nervous system. Inducible NOS, which is synthesized in neurons, vascular cells, and glia under various pathologic conditions, produces NO independently of calcium concentrations (Veltkamp et al, 2002). The relevance of nitric oxide was increased with the report that the diffusion-limited reaction between superoxide and nitric oxide gives rise to peroxynitrite (Beckman et al., 1990). The highly reactive peroxynitrite provided a mechanistic basis for oxidative stress derived from...
increased nitric oxide production caused by ischemia/reperfusion (Eliasson et al., 1999). Nitric oxide has also been shown to inhibit mitochondrial respiration via competition with oxygen for cytochrome oxidase (Brown and Borutaite, 1999) and play a role in the initiation of apoptosis (Bonfoco et al., 1995). Although little has been reported on efforts to bring nitric oxide inhibitors to clinical investigation, there is no doubt that nitric oxide plays a pivotal role in mediating oxidative stress (Mikkelsen and Wardman, 2003).

**Drugs used in our studies**

**S-allyl cysteine**

Empirical Formula: C₆H₆NO₅S

Molecular weight: 166, 25

**Description**

S-allyl cysteine (SAC) is the most abundant organosulfur compound in garlic extract with potential antioxidant and antiinflammatory properties (Maldonado et al., 2003). Since oxidative damage is implicated in the etiology of neurological complications, treatment with antioxidants has been used as a therapeutic approach in various types of neurodegenerative disease. The therapeutic effects of SAC were assessed in various models of neurodegenerative diseases including stroke (Kim et al., 2006; Atif et al., 2009), Alzheimer disease (Pérez-Severiano et al., 2004; Javed et al., 2011), and Parkinson disease (García et al., 2010). The molecular mechanisms of these effects may include protective actions through its ability to scavenge $O_2^-$ and $H_2O_2$, thus preventing $H_2O_2$-induced endothelial cell damage and lipid peroxidation, as well as low-density lipoprotein oxidation (Ide and Lau, 2001), mitochondrial damage, and subsequent cell death. S-allyl cysteine also reduces edema formation in the ischemic rat brain through the inhibition of LPO (Numagami et al., 1996) and produces neuroprotective effects on the amyloid-beta peptide-induced oxidative damage, and learning deficits (Peng et al., 2002).
Catechin Hydrate

Empirical Formula: C_{15}H_{14}NO_6 \cdot xH_2O
Molecular weight: 290, 27

Description
Catechin is a polyphenolic flavonoid which has been isolated from a variety of natural sources including green tea, grape seeds, and the wood and bark of trees such as acacia and mahogany (Alshatwi, 2010). Green tea (Camellia sinensis) has been used as a medicine for thousands of years (Sutherland et al. 2006). Catechin hydrate is a more potent antioxidant that scavenges radicals; suppress lipid peroxidation, exhibit neuroprotective effect on ischemic stroke (Sutherland et al. 2006). Experimental studies suggest that catechin hydrate slow the atherogenic process (Kaliora et al., 2006; Gendron et al., 2010), as well as the decline in the learning ability associated with brain hypoperfusion. The neuroprotective effects of many polyphenols rely on their ability to permeate brain barrier and here directly scavenge pathological concentration of reactive oxygen and nitrogen species and chelate transition metal ions (Aquilano et al 2008). When green tea is given to rats, its polyphenols can cross the blood-brain barrier and display neuroprotective effects, partly by improving the endothelial function via a reduction in lipid peroxidation (Haque et al., 2006). A recent clinical study suggests that the daily consumption of green tea reduces the risk of stroke (Sato et al., 1989; Arab et al., 2009). Different polyphenolic compounds were shown to have scavenging activity and the ability to activate key antioxidant enzymes in the brain and thus breaking the vicious cycle of oxidative stress and tissue damage (Esposito et al 2002). Green tea possesses anticancer potential and is one of the most commonly used herbal medicines worldwide (Al-Hazzani and Alshatwi, 2011). Moreover, epidemiological observations have suggested that the consumption of green tea exhibit antiangiogenic properties, antiplatelet activity and inhibits growth of many tumor types (Prior and Cao, 1999; Katiyar and Elmets, 2001). Attenuation of the release of cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and NF-kB was also ascriptive to different polyphenols of green tea (Vafeiadou et al., 2007). Thus, natural polyphenolic compounds may protect and/or improve physiological brain functions by different mechanisms that include mainly antioxidant activity, Nitric oxide synthase balance, and inhibition of endothelin production and attenuation of cytokine release. We therefore,
hypothesized that a chronic catechin hydrate treatment would protect the cerebral Ischemia reperfusion injury and related brain disorder.

**Phloretin**

Empirical Formula: $\text{C}_{15}\text{H}_{14}\text{O}_5$

Molecular weight: 274, 27

![Fig. 1.18 Structure of Phloretin](image-url)

**Description**

Phloretin is a dihydrochalcone flavonoid found exclusively in apples and in apple-derived products and pears. It is present as free and its glucosidic form, phloridzin (phloretin 2-O-glucose) (Shao et al., 2008; Jugde et al., 2008). In addition to extraction from apples, now a days phloretin can be prepared easily from the available compound dihydrochalcone (DHC). It has many biological and pharmacological properties, such as potent antioxidant activity in peroxynitrite scavenging, inhibition of lipid peroxidation (Vasantha and Yasmin, 2010), antiproliferative effects (Rezk et al., 2002). It has been shown to exert antitumor activity through inhibition of protein kinase C (PKC) activity and induction of apoptosis (Kern et al., 2007). However, little is known about the protective mechanism by which phloretin rescues cells from oxidative stresses. Along with its glucoside phloridzin, phloretin is believed to be an important contributor to the health benefits. It was reported that phloridzin could be hydrolyzed to phloretin by lactase-phloridzin hydrolase in the small intestine (Crespy et al., 2001). A more recent study showed that urinary phloretin could be used as the exposure biomarker of apple consumption (Mennen et al., 2006). Phloretin has been reported to prevent cytokine-induced expression of endothelial adhesion molecules and to reduce activation of human platelets (Hassan et al., 2007). Over the past few years, much attention has been focused on flavonoids, a group of phenolic compounds that have been linked to a reduced risk of major acute as well as chronic diseases including neurodegenerative diseases (Arts and Hollman, 2005).
Thymoquinone (TQ)

Empirical Formula: C_{10}H_{12}O_{2}
Molecular weight: 164, 2

Description

Nigella sativa L., commonly known as black seed or black cumin, is used in folk medicine as a natural remedy for a number of diseases and conditions such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, and gastrointestinal disturbances (Ali and Blunden, 2003). Thymoquinone (TQ) is the bioactive constituent of the volatile oil of nigella sativa and has been shown to exert antioxidant, antineoplastic, and anti-inflammatory effects (Houghton et al., 1995; Abdel-Fattah et al., 2000) blocked pancreatic cancer cell growth and killed the cells by enhancing the process of programmed cell death (apoptosis). In Islam, it is regarded as one of the greatest forms of healing medicine available. Prophet Muhammad (SAW) once stated that the black seed can heal every disease—except death. TQ, active constituent of N. sativa seeds, is a pharmacologically active quinone, which possesses several properties including protects organs against oxidative damage induced by a variety of free radical generating agents including doxorubicin induced cardiotoxicity (Nagi and Mansour, 2000). The volatile oil of N. sativa was shown to contain about 24% thymoquinone (Arslan et al., 2005). Thymoquinone acts as a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen (Badary et al., 2003). TQ has also exerted neuroprotective effects against transient forebrain ischemia and MPP+ and rotenone model of Parkinson’s disease in rat (Al-Majed et al., 2006; Radad et al., 2008).

Although TQ is a powerful antioxidant and anticancer drug, its administration is limited due to poor water solubility (Gali-Muhtasib et al., 2006), leading to low absorptivity and bioavailability. In addition, administration of high dosages to rats has resulted in hypoactivity and difficulty in respiration associated with reduced glutathione in the liver and kidney (Badary et al. 1997). Therefore, novel antioxidant-loaded drug delivery systems such as polymeric nanoparticles have been identified as alternatives. To overcome these disadvantages, biodegradable and biocompatible polymeric nanoparticles would be attractive alternatives for TQ delivery. Such nanoparticles would likely provide improved TQ solubility, controlled delivery, and enhanced therapeutic properties.

* * * * *