Review of Literature
Review of Literature

To those of us who are humbly exploring the mysteries of science, we must project our findings and model our systems. If correct, we have made a small contribution and, if wrong, we have forced others to eventually think. The aim of this review is to clarify certain aspects of cerebral ischemia that are relevant to the studies in the thesis and it has no ambition of being complete.

Stroke is the third leading cause of mortality worldwide. More than two-thirds of stroke deaths occur in the developing world. Throughout the world, unfavorable trends in stroke risk factor profile portend high stroke rates and serve to widen the stroke prevention gap. This is unfortunate because stroke is well suited for prevention since it has a high prevalence, high burden of illness and economic cost, well-defined modifiable risk factors, and effective preventive measures (Gorelick 2002). Annual risk assessment, screening and intervention should be part of a concerted national effort to reduce the incidence of the third leading cause of death and the number one cause of adult disability. Many workers portend that cost of rehabilitation of stroke-victims in India, will pose enormous socio-economic burden on our meager health care resources in a similar manner what is now faced by the developed nations of the West.

Present demographic trends suggest that the Indian population will survive through the peak years of stroke occurrence (55-65 yr) and the degree of the residual morbidity will pose a major medical problem. Community surveys from many regions of India show a crude prevalence rate for strokes presumed to be of vascular origin in the range of 200 per 100,000 persons.

HISTORY OF STROKE

Hippocrates, the father of medicine, first recognized stroke over 2,400 years ago. At that time stroke was called apoplexy, which means "struck down by violence" in Greek. This was due to the fact that a person developed sudden paralysis and change in well-being. Physicians had little knowledge of the anatomy and function of the brain and the cause of stroke, or how to treat it. It was Galen (AD 138-201) who described the important carotid arteries and intracranial vessels. It was not until the mid-1600s that Jacob Wepfer found that patients who died with apoplexy had bleeding in the brain. He also discovered that a blockage in one of the brain's blood vessels could cause apoplexy. Medical science continued to study the cause, symptoms, and treatment of apoplexy and finally in 1928, apoplexy was divided into categories based on the cause of the blood vessel problem.

![Figure 1: An Embolus in the Bifurcation of the Middle Cerebral Artery.](image)

This led to the terms stroke or "cerebral vascular accident (CVA)". Stroke is now often referred to as a "brain attack" to denote the fact that it is caused by a lack of blood supply to the brain, very much like a "heart attack" (Figure 1).

MAJOR CEREBRAL ARTERIES

Our brain needs a constant supply of oxygen-rich blood to nourish its 100 billion
nerve cells. When this supply is reduced or shut off completely, starving brain cells die within minutes. That is why stroke is sometimes called a “brain attack”. The pertinent organ, brain receives 25% of the body’s oxygen supply but cannot store it and yet the brain cells need a constant supply for their survival and healthy functioning. For which the blood needs to be continuously supplied to the brain, which is done through two main arterial systems.

- **The carotid arteries** come up through either side of the front of the neck. (The pulse of a carotid artery can be felt by placing the fingertips gently against either side of the neck right under the jaw) (Figure 2 and 3).
- **The basilar artery** forms at the base of the skull from the vertebral arteries, which runs up along the spine, join, and come up through the rear of the neck.

![Diagram of arterial structures]

**TYPES OF STROKE**

Strokes can be classified into two main categories, including the following (Figure 4):

- **Ischemic strokes** - strokes caused by blockage of an artery.
- **Hemorrhagic strokes** - strokes caused by bleeding.

**Ischemic Stroke**

An ischemic stroke occurs when a blood vessel that supplies the brain becomes blocked or “clogged” and impairs blood flow to part of the brain. The brain cells and tissues begin to die within minutes from lack of oxygen and nutrients. The area of dead tissue is called an infarct. About 80% of strokes fall into this category (Figure 5). Ischemic strokes are further divided into two groups, including the following:
Thrombotic Strokes—This specific type of stroke is caused by a thrombus (blood clot, pieces of calcium and fatty plaques) that lodges in the blood vessels inside the brain. Thrombus formation involves various steps from its pathology, arterial wall injury, platelet formation, plaque fissuring to the evolution of thrombus formation. It occurs in older persons with high cholesterol and atherosclerosis. Sometimes occurrence is sudden and gradually evolves; this is called as “stroke in evolution” and precedes mini strokes called as “transient ischemic attacks (TIA’s)”. They are considered as warning signs or biomarkers of stroke. About 5% TIA sufferers experience stroke within a month, and without treatment, a third will have strokes within five years. One of two major arteries is usually involved in a transient ischemic attack, either the carotid or basilar arteries. Another in this list is lacunar infarct, which occurs in small blood vessels and is found in people with diabetes and hypertension.

Embolic Strokes—It is caused by an embolus (a blood clot that develops elsewhere in the body and then travels to one of the blood vessels in the brain via the bloodstream) result from heart diseases/surgeries, rapidly and without warning signs. About 15% causes appear in people with atrial fibrillation (abnormal heart beat in the upper chambers of heart).

Hemorrhagic Stroke

Hemorrhagic stroke occur when a blood vessel that supplies the brain ruptures and bleeds. When an artery bleeds into the brain, brain cells and tissues do not receive oxygen and nutrients. Pressure builds up in the surrounding tissues, causing irritation and swelling. About 20% of strokes are due to hemorrhage (Figure 6). Hemorrhagic strokes are divided into the following two main categories

Intracerebral hemorrhage—When bleeding from the blood vessels within the brain occurs suddenly and rapidly. It is usually seen in hypertensive patients with no warning signs. Bleeding may be too severe to cause coma or death.

Subarachnoid hemorrhage—Often due to aneurysm (a weakened, ballooned area on an artery wall with risk for rupturing, they may be congenital or develop later in life) or arteriovenous malformation (AVM, a congenital disorder of a disorderly tangled web of arteries and vein, cause is unknown) consequently bleeding in the subarachnoid space takes place (the space between the
Review of literature

brain and the membranes that cover the brain).

RECURRENT STROKES

Recurrent strokes occur in about 25% of stroke victims within five years after a first stroke. The risk is greatest right after a stroke and decreases over time. The likelihood of severe disability and death increases with each recurrent stroke. About 3% of stroke patients have a second stroke within 30 days of their first stroke and about one-third have a second stroke within two years.

SILENT BRAIN INFARCTIONS

As many as 31% of the elderly experience silent brain infarctions, which are small strokes that cause no apparent symptoms but are major contributors to mental impairment. Smokers and people with hypertension are at particular risk.

PATHOGENESIS And PATHOPHYSIOLOGY

Pathology: The pathogenic processes leading to thrombus formation are complex and many of the intermediary steps are not completely understood. In the brain, the process is better characterized in the larger arteries than in small arteries supplying deep cerebral white matter. Atherosclerosis is a decade-long process in which the lumen of a blood vessel becomes narrowed by cellular and extracellular substances to the point of obstruction. Fatty streaks, which are grossly visible as areas of yellowish discoloration on the surface of the intimal layer of the vessel wall, are widely distributed throughout the coronary arterial vasculature. On microscopy, the lesions primarily consist of lipid-filled macrophages (foam cells). Atheromatous lesions evolve into complicated fibrous plaques, consisting of a central acellular area of lipid covered by a cap of smooth muscle cells and collagen. Caps tend to form slowly at first, but with deposition of platelets and fibrin on the surface, which appears to be the result of endothelial injury the caps thicken quickly, as a result of thrombosis-dependent fibrotic organization. The progression of early atherosclerotic lesions to clinically relevant advanced atherosclerotic lesions occurs with increased frequency in persons with risk factors for atherosclerotic disease (e.g., hypercholesterolemia, hypertension, cigarette smoking). When lipoproteins are accumulated in the artery wall over a prolonged period, (hypercholesterolemia), the antioxidants are depleted (Stephans et al. 1996).

Figure 7: Showing the yellow fatty streaks (foam cells) on the vessel wall.

LDL uptake in sub-endothelial macrophages and smooth muscle cells is strictly regulated by the LDL-receptor, but oxidized LDL (oxy-LDL) is abundantly incorporated into sub-endothelial macrophages by an unregulated "scavenging" receptor or phagocytosis (Berkel et al. 1991). Overloaded cells with oxy-LDL molecules (the "foam cells") form the basis of atheromatous plaques in the artery wall (Figure 7) (Steinberg 1993). Concomitantly, oxy-LDL blocks cholesterol uptake by HDL molecules and promotes platelet's adhesion to endothelium, which initiates a complex cellular reaction leading to the development of atherosclerotic lesions (Elwood et al. 1991, Renaud and De-Lorgeril 1992). The atherogenic modifications of lipoproteins are accompanied by the enhanced blood platelet's adhesion and aggregation, and increased expression of tissue factor (TF),
Review of literature

which is a primary initiator of coagulation; not expressed by monocytes-macrophages and endothelial cells. Its inappropriate appearance on these cells surfaces triggers blood clotting. TF accumulates to a great extent in elements which form human atherosclerotic plaques, macrophages, smooth muscle and endothelial cells and in cell-free, cholesterol-rich layers. It is thought to determine plaque thrombogenicity, contractile functions and organ failure (Kaul et al. 1993, Semeraro and Colucci 1997).

Arterial Wall Injury In Thrombus Formation

Atherosclerosis begins in response to chronic minimal injury to the endothelium (the continuous monolayer of cells lining the arterial wall) and the interactions among monocytes, lipoproteins, platelets, lymphocytes and smooth muscle cells abet and continue the pathogenic process (Figure 8). Intima, which lies below the endothelium, is the main battleground of the atherosclerotic process. The media and the adventitia also play their role as the development of atherosclerosis. It is characterized by accumulation of complex lipids, proteins, and carbohydrates as well as proliferation of cells in the intimal layer of an artery.

Plaque Fissuring And Formation

The mechanisms of plaque destabilization (fissuring and rupture, followed by thrombus formation) are not fully understood. Study of plaques in the coronary arteries that 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, as well as infiltration of the cap tissue with foam cells (with the reduction of total collagen content and a concomitant fall in tensile strength).

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.

SYMPTOMS OF STROKE

Persons at risk or caretakers of persons at risk should be aware of the general symptoms so that the stroke victims are hospitalized soon after the warning signs. It is reported that 30% of the stroke cases were neither evaluated nor sent to the hospital within a month after the first event.

Symptoms of Cerebral Ischemia

➤ With oxygen to the eye reduced, there is blurring of vision.
➤ Person experiences problems in speech, tingling, numbness, partial and temporary paralysis usually on one side of the body.
➤ Abrupt headaches.
➤ Nausea and vomiting.
➤ Sensitivity to light.
➤ Neck stiffness.
➤ Altered states of consciousness.
➤ Stupor, rigidity, and coma.
Review of literature

- Dizziness.
- Difficulty swallowing.
- Weakness in the arms and legs, sometimes causing a sudden fall.
- Altered mental states.

Effects of Stroke

Figure 9: Illustrating various brain regions affected during stroke.

<table>
<thead>
<tr>
<th>Region of the Brain Damaged by Stroke</th>
<th>Signs &amp; Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wernicke's area (central language area)</td>
<td>Difficulty in speaking understandably, reading, writing, naming objects, &amp; calculating, comprehending, confusion between left and right</td>
</tr>
<tr>
<td>Broca's area (speech)</td>
<td>Difficulty speaking and, sometimes, writing</td>
</tr>
<tr>
<td>Parietal lobe on the left side of the brain</td>
<td>Loss of coordination of the right arm and leg</td>
</tr>
<tr>
<td>Facial &amp; limb areas of the motor cortex on the left side of the brain</td>
<td>Paralysis of the right arm and leg and the right side of the face</td>
</tr>
<tr>
<td>Facial arm areas of the sensory cortex</td>
<td>Absence of sensation, visual field (right side), Optic radiation</td>
</tr>
</tbody>
</table>

Table showing effects of stroke.

Risk Factors for Stroke

New and recurrent strokes affect about 600,000 people every year. Incidence of stroke has increased and also the survival rate. Various risk factors of stroke are as follows:

Age

Older Adults: Older adults particularly with high blood pressure, sedentary habits, overweight, diabetic and those who smoke are at increased risk. Studies are mixed on the effects of stroke by gender.

Younger Adults. Not at risk, still 25% of stroke victims are below 65.

Gender

Regardless of the ethnic groups and mixed studies, ischemic strokes are more deadly in men than in women. High risks for hemorrhagic strokes and atherosclerosis reported, though the increased vulnerability in men has not been marked out.

Ethnicity

There is great disparity among the ethnic groups regarding stroke prevalence. Native Americans, Hispanics, and African Americans are at an increased risk than American Caucasian's. Reasons attributed to these differences are:

- African Americans have a higher prevalence of diabetes and hypertension than other groups.
- Poor diets, high stress levels and lack of health care accessibility certainly play their role.
- A study confirmed that African Americans produce less nitric oxide in response to stress; this substance is critical for opening blood vessels and increasing blood flow.
- Strokes are more common in the Southeastern U.S. (the so called “Stroke Belt”) than in other areas. Reasons for this geographical variation are unclear.

High Blood Pressure (Hypertension)
This factor is the major contributor of 70% of all strokes. Of all the known cases of strokes, controlling blood pressure can avert 405 cases. Two variables are used to describe blood pressure and affect stroke risk separately:

**The systolic pressure** (higher and first number): Elevated systolic pressure poses an increased threat when diastolic is normal a condition called isolated systolic hypertension. The wider the spread between systolic and diastolic the greater the difference.

**The diastolic pressure** (lower and second number): Abnormally higher diastolic pressure is a strong predictor of heart attack and stroke in most people with hypertension.

**Atrial Fibrillation**

About one in six stroke is due to atrial fibrillation, a heart rhythm disorder in which the atria (the upper chambers in the heart) beat very quickly and ir rhythmically. The blood pools, instead of being pumped out, increases the risk for formation of blood clots that break loose and travel toward the brain. Atrial fibrillation, in fact, poses a six-fold increased risk for stroke and may also pose a higher risk for complications after a stroke. Atrial fibrillation is uncommon in people under 60 years old, but about 6% of adults over 80 have this heart rhythm disorder.

**Smoking**

Two and a half times increased risk in smokers than in non-smokers, poses an increased risk for both hemorrhagic and ischemic stroke.

**Diabetes and Insulin Resistance**

A putative risk factor, if the insulin levels are high the body is unable to use insulin normally to metabolize sugar. The body compensates by raising the level of insulin (hyper-insulinemia), which can, in turn, increase the risk for blood clots and reduce HDL levels (the beneficial form of cholesterol).

**Obesity and Sedentary Lifestyles**

Increased risk factor for both hemorrhagic and ischemic stroke has coexistence with insulin resistance and diabetes, high blood pressure and unhealthy cholesterol level. Weight centered on the abdomen (called apple shape) has a particularly high association with stroke, as it does for heart disease, in comparison to weight around hips (pear shape).

**Cholesterol and Other Lipids**

Role of lipids in stroke is still not clear. Different lipids have different effects:

**Ischemic stroke.** HDL (the so-called good cholesterol) may be the most important lipid for preventing ischemic stroke. The effects of high total cholesterol and LDL levels on stroke are less clear. Studies have suggested that the risk for ischemic stroke increases when total cholesterol is above 280 mg/dl.

**Hemorrhagic Stroke.** HDL may also reduce the risk for hemorrhagic stroke. People with overall cholesterol levels below 180 mg/dl, however, may be at risk for hemorrhagic stroke, particularly if they also have high blood pressure. This is a fat less common stroke than ischemic stroke. In any case, reducing cholesterol is extremely important for anyone with heart disease and abnormal lipid profile.

**Alcohol, Coffee and Drug Abuse**
**Review of literature**

*Alcohol*: Heavy alcohol use is associated with increased risk of stroke. Studies however, indicate that a moderate drinker has significantly lower risk for ischemic stroke, although not hemorrhagic stroke.

*Coffee*: Having 3 or more cups poses a threat in older age with persons already having hypertension.

*Drug Abuse*: Particularly with cocaine and increasing methamphetamine, is a major risk factor in young adults. Steroids used for bodybuilding also increase the risk.

**Genetic and Inborn Factors**

Genetics may be responsible for many of the causes of stroke. Studies indicate that a family history of stroke, particularly in one’s father, is a strong risk factor for stroke.

**Mental and Emotional Factors**

*Stress*: Surveys reveal that prolonged, intense or frequent mental stress causes an exaggerated increase in blood pressure, which is the major cause of stroke.

*Depression*: Depression has also been linked to a higher risk for having a stroke and lower survival rates after one. Patients with severe depression had a 73% higher risk for stroke, and those with moderate depression had a 25% higher risk than average.

*Migraine and Associated Risk Factors*

Studies have shown that migraine or severe headache is a risk factor for stroke in both men and women, especially below age 50. In fact, migraine is associated with about 19% of all strokes. Oral contraceptives add to the risk for stroke in young women with migraines, but only in those who also have auras. Smoking and taking decongestants intensifies their risk.

Reports have indicated for some time that certain bacteria and viruses may play a role in atherosclerosis, heart disease and stroke, provoke an inflammatory response in the arteries.

*Other Factors Associated with Stroke*

*Timing*: Stroke appears to be more common in morning hours, perhaps due to temporary rise in blood pressure at that time. Various studies point out that during holidays and in winters the risk is more.

*Height*: Shorter people are at higher risk than taller individuals.

*Homocysteine and Vitamin B deficiencies*: Abnormally high blood levels of the amino acid homocysteine, which occur with deficiencies of vitamin B6, B12, and folic acid, are strongly linked to an increased risk of coronary artery disease and stroke. These are considered as a chemical bystander, which increases in the presence of other risk factors.

**SYSTEMS USED TO STUDY ISCHEMIA**

Many experimental models have been used to study ischemic damage. Mechanisms of cell damage are determined by testing effects of different manipulations on the extent of cell death in a model. To meaningfully compare results between models, the essential differences between the ischemic insults and the way the damage is assessed in these models needs to be appreciated. Three main classes of in vivo rodent models of ischemic study are global ischemia, focal ischemia and hypoxia/ischemia. In the latter, vessel occlusion is combined with breathing a hypoxic mixture.

**GLOBAL ISCHEMIA**

Global ischemic insults are most commonly produced by vessel occlusion, and less common by complete circulatory arrest.
Though the former is not actually global, a large portion of the forebrain is quite uniformly affected. The three most widely studied global ischemic models are four-vessel occlusion (4-VO) and two-vessel occlusion (2-VO) combined with hypotension in the rat, and two-vessel occlusion in the gerbil. It is widely used because of the relative ease of the surgical techniques involved. Complete global ischemia can be achieved by neck-cuff, cardiac arrest, or by ligating or compressing all arteries stemming from the heart. Blood flow to then whole brain is zero or <1% in these models.

FOCAL ISCHEMIA

This model mimics human stroke and is widely used even though the surgical technique involved is much complex than global ischemia. It may be permanent or transient period. In permanent, the arterial blockage is maintained throughout the experiment, usually 1 to several days. In transient or temporary focal models, vessels are blocked up to 3 h, followed by reperfusion. Both permanent and transient models are of value in examining different aspects of the extent of damage. Different sites and techniques of artery occlusion are used to obtain reproducibility of flow reduction and lesion size. There are two principle occlusion sites. Occlusion of the middle cerebral artery provides the most widely used approach, which generates tissue infarction comparable as seen in humans (Lipton 1999, Sims and Anderson 2002).

In proximal occlusion, the middle cerebral artery is occluded close to branching from the internal carotid. A newer and widely used approach to proximal MCA occlusion is the insertion of a nylon suture into the carotid artery past the point at which the MCA branches so that the latter is occluded at its origin. In distal MCA occlusion, flow to the basal ganglia is not damaged so that the damage is only cortical. The occlusion can be made surgically, in which the lesion is readily reversible or less invasive by creating a thrombus by laser irradiation when the artery is perfused with Rose Bengal. Distal MCA has to be combined with occlusion of the ipsilateral carotid artery to get adequate flow reduction.

How serious are strokes?

A stroke is “always” serious. The mortality rates are declining as 80% of patients survive a stroke attack. But the % age of morbidity is increasing due to lack of rehabilitation programs after stroke. In the western countries, the incidence of CVD is reported to be 500-800 per 100,000 population per year. Precise data of similar type are not available for India but some reports indicate the incidence to be 13-33 per 100,000 population per year (Viriyavejakul 1994, Banerjee 2000). These figures are definitely lower as compared to the western countries, but considering the total population of India, numerically the problem is perhaps greater than most of the other countries.

Ischemic vs. Hemorrhagic Stroke

People who suffer from ischemic strokes have much better chance for survival than those who experience hemorrhagic strokes. Among the ischemic stroke categories, the greatest dangers are posed by embolic strokes, followed by large-artery (thrombotic) and lacunar strokes. Hemorrhagic stroke not only destroys brain cells, but it poses other complications as well, including increased pressure on the brain or spasms in blood vessels, both of which can be very dangerous.

Factors Affecting Recurrence

The risk for recurring stroke is highest within the first few weeks and months. The risk is about 10% in the first year and 5% hereafter, so preventive measures should be
Review of literature

instituted as soon as possible. Some specific risk factors for early recurrence are the following:
Being older, evidence of blocked arteries, being diabetic, alcoholism, valvular heart disease, atrial fibrillation.

Long-Term Outlook for Survivors

Over half of stroke victims survive beyond five years. Between 50%-70% of stroke sufferers regain functional independence. Between 15%-30% of Those who survive either an ischemic or hemorrhage strokes suffer some permanent disability. On the encouraging side, about 90% of stroke survivors experience varying degrees of improvement after rehabilitation.

Rehabilitation Approaches

Physical therapy should be started as soon as the patient is stable. Since stroke affects different parts of the brain, specific approaches to managing rehabilitation vary widely among individual patients.

- Physical exercise (retraining muscles) relating to the disability caused by the stroke is, in any case, important and may actually help repair the brain.
- While professional speech therapy progresses, the patient's caregivers should use and encourage the patient in non-verbal communications, such as pantomime, facial expressions, pen and paper. Learning and using the sign-language alphabet may be helpful both in communicating and improving small-motor dexterity. Biofeedback techniques combined with physical therapy should be employed Electrical stimulation of the throat, for example, may help patients with dysphagia recover their ability to swallow faster. Stimulation of the wrist and finger is also showing promise for improving motor capabilities. About 30% of patients experience aphasia (an impaired ability to speak), which is particularly distressing. It is necessary to understand that this disability does not necessarily impair the ability to think. Although confusion is common among people who have had strokes, partial or even complete recovery is very possible.
- Problems in attention are very common after strokes. A variant of this approach trains patients to relearn real-life skills, such as driving, carrying on a conversation or other daily skills.

Evaluation procedures and various diagnostic tests for Stroke

Evaluating and diagnosing damage to the nervous system is complicated and complex. Many of the same symptoms occur in different combinations among different disorders. To further complicate the diagnostic process, many disorders do not have definitive causes, markers/tests. To save a patient's life, a fast diagnosis of both the presence and type of stroke is critical. The first step is to determine whether symptoms actually indicate a stroke. (Simple verbal and physical tests enable emergency teams to identify nearly in all stroke patients.) Non-invasive techniques for diagnosing transient ischemic attacks are also used for major strokes. In addition to a complete medical history and physical examination, diagnostic procedures for nervous system disorders may include the following:

IMAGING TESTS OF THE BRAIN

Computed tomography (CT or CAT scan): It is a diagnostic imaging procedure that uses a combination of x-rays and computer technology to produce cross-sectional images of the body (often called slices), both horizontally and vertically. A CT scan shows detailed images of any part of the body, including the bones, muscles, fat, and organs. CT scans are more detailed than general x-rays; used to detect
abnormalities and help identify the location or type of stroke.

**Magnetic Resonance Imaging (MRI):** MRI is a diagnostic procedure that uses a combination of large magnets, radio frequencies, and a computer to produce detailed images of organs and structures within the body. It detects small changes in brain tissue that helps to locate and diagnose stroke (Figure 10).

![Figure 10: Magnetic resonance imaging in acute stroke. Diffusion-perfusion mismatch in acute ischemic stroke. The perfusion abnormality (right) is larger than the diffusion abnormality (left), indicating the ischemic penumbra, which is at risk of infarction.](image)

**Radionuclide Angiography**

It is a nuclear brain scan technique in which radioactive compounds are injected into a vein in the arm and a machine (similar to a Geiger-Müller counter). It produces a map showing their uptake into different parts of the head. The images show the nature of brain functions rather than its structure. This test can often detect areas of decreased blood flow and tissue damage.

**TESTS THAT EVALUATE THE BRAIN'S ELECTRICAL ACTIVITY**

**Electroencephalogram (EEG):** ECG is a procedure that records the brain's continuous electrical activity by means of electrodes attached to the scalp.

**Evoked Potentials:** It is a procedure that records the brain's electrical response to visual, auditory and sensory stimuli. Electro Diagnostic Tests (i.e., electromyography (EMG) and nerve conduction velocity (NCV)): These are the studies that evaluate and diagnose disorders of the muscles and motor neurons. Electrodes are inserted into the muscle, or placed on the skin overlying a muscle or muscle group, thus recording electrical activity and muscle response.

**Positron Emission Tomography (PET):** In nuclear medicine, PET is a procedure that measures the metabolic activity of cells.

**Arteriogram (Angiogram):** These are the additional scanning of the arteries and a vein by x-ray's to detect blockage or narrowing of the vessels (Figure 11).

![Figure 11: Showing carotid angiogram.](image)

**Neurosonography:** As the name indicates, it is a procedure that uses ultra high-frequency sound waves to enable the physician to analyze blood flow in the cases of possible stroke.

**Ultrasound (Sonography):** This diagnostic imaging technique uses high-frequency sound waves and a computer to create images of blood vessels, tissues and organs. Ultrasounds are used to view internal organs as they function and to assess blood flow through various vessels.

**Tests that measure blood flow**

**Carotid Phonoangiography:** In this procedure a small microphone is placed over the carotid artery on the neck to record sounds created by blood flow as it passes.
through a partially blocked artery. The abnormal sound is called a bruit.

**Doppler Sonography:** Here a special transducer is used to direct sound waves into a blood vessel to evaluate blood flow. An audio receiver amplifies the sound of the blood moving through the vessel. Faintness or absence of sound may indicate a problem with blood flow.

**Ocular Plethysmography:** It measures pressure, or detects pulses in the eyes.

**Cerebral Blood Flow Test (inhalation method):** The amount of oxygen in the blood supply that reaches different areas of the brain is measured by this technique.

**Digital Subtraction Angiography (DSA):** It provides an image of the blood vessels in the brain detecting any problem with blood flow. The test involves inserting a small thin tube (catheter) into an artery in the leg and passing it up to the blood vessels in the brain. A contrast dye is injected through the catheter and x-ray images are taken.

**HEMATOLOGICAL TESTS**

- Blood tests are used to determine clotting times.
- Blood sugar levels (hyperglycemia) are measured.
- Glutamate, an amino acid measurement.
- The inflammatory response in the brain stimulates the release of certain markers that are used to diagnose a stroke. C-reactive protein, enzymes called troponins and elevated erythrocyte sedimentation rates (ESR) are also indicators of the inflammatory process and may predict a higher risk for stroke and a poorer outcome in people with existing stroke.
- Elevated levels of lipoprotein (a) may reveal the possibility of an unruptured aneurysm, which can be confirmed with an MRI (magnetic resonance imaging).

**TYPES OF SURGERY TO TREAT OR PREVENT A STROKE**

Several types of surgery may be performed to help treat a stroke, or help to prevent a stroke from occurring, including the following:

**Carotid Endarterectomy**

It is a procedure used to remove plaque and clots from the carotid arteries, located in the neck. These arteries supply the blood to the brain from the heart. Endarterectomy may help to prevent a stroke from occurring.

**Craniotomy**

A craniotomy is a type of surgery in the brain itself to remove blood clots or repair bleeding in the brain (Figure 13).

**Figure 12:** Showing carotid endarterectomy.

**Figure 13:** White Platelet–Fibrin Material (Arrow) in the cortical branch of the middle cerebral artery flowing from left to right and exposed at craniotomy in a patient undergoing superficial anastomosis between the temporal and middle cerebral arteries (MCA) (X 10)
Surgery To Repair Aneurysms And Arteriovenous Malformations (AVMs)

An AVM is a congenital or acquired disorder that consists of a disorderly, tangled web of arteries and veins. An AVM also has a risk for rupturing and bleeding into the brain. Surgery may be helpful, to help prevent a stroke.

**DRUG THERAPY FOR REHABILITATION**

Drug therapy can sometimes help relieve specific effects of stroke:

- Dantrolene (Dantrium), baclofen, and injections of the deadly bacterial toxin, botulism, have shown some promising effect in relieving spasticity.
- The drug bromocriptine (Parlodel), normally used for Parkinson's disease, was helpful for patients with severe speech problems, improving their ability to pronounce multi-syllable words and to form sentences.
- Some patients experience intractable hiccups, which can be very serious. Among the drugs used for this condition are chlorpromazine or baclofen.
- The use of amphetamines may help improve speech and motor skills when combined with physical therapy.

**Drugs Used To Treat Stroke Patients**

*Intravenous Thrombolitics*

Thrombolitics or clots busting drugs are given to patients who suffer from embolic or hemorrhagic stroke (increased risk of bleeding). The standard drug of this category is t-PA (tissue plasminogen activator) or alteplase (Activase) is injected directly into an artery in the brain. Critical investigations are required prior to its administration. e.g.,

- A CT to confirm that the stroke is not hemorrhagic.
- To be administered within 3 hr of stroke not after that, to have an effect.
- Never administered in hemorrhagic stroke because of the increased risk of bleeding.

**Ancredo**: An agent derived from the venom of a pit viper snake that reduces blood clotting factor, fibrinogen. It is a proposed alternate to thrombolytics, administered within 3 hr. With all anti-clotting agents there is an increased risk for hemorrhage.

Anti-Clotting Medications: Drugs that prevent blood clotting have been given to patients who are at risk for recurrent stroke. Treatment reduces the chances of second stroke, heart attack, and related deaths by 22%. Time window is 48 h after ischemic stroke and continued as maintenance. The specific anti-clotting agents are generally recommended in the following order:

**Aspirin**: A number of trials found that aspirin could prevent a first ischemic stroke. It has modest effect in preventing a second stroke and is the most widely recommended agent as initial therapy in doses of between 50-325 mg. It is not clear if aspirin should be used after a first stroke in patients who have been taking it before the stroke for heart attack prevention or other medical problems.

**Aggrenox**: A single capsule containing both low-dose aspirin and extended release dipyridamole, an anti-platelet agent. Their complementary actions are more effective than ordinary aspirin for preventing a second stroke in high-risk people; the drug also has a good safety profile. Much more expensive than aspirin, however, recommended only if aspirin does not appear to be helpful.

**Thienopyridines**: Ticlopidine (Ticlid) or clopidogrel (Plavix) are anti-blood platelet agents known as thienopyridines. Slightly
more protective against stroke than aspirin, but they are costly. They are options for patients who cannot tolerate aspirin. These agents, however, can have severe side effects. Ticlopidine particularly has been associated with reversible lupus-like symptoms (an autoimmune disease), reversible neutropenia (a drop in white blood cells), and thrombocytopenia (a severe drop in platelet counts). Clopidogrel has been preferred because of its better safety record, but reports of thrombocytopenia in patients taking clopidogrel have created concern.

**Warfarin:** This anticoagulant is a potent anti-clotting agent and needs to be monitored carefully as it can lead to bleeding. It can be efficient in patients with atrial fibrillation or high-risk patients who do not respond to other anti-platelet drugs. All anti-clotting drugs carry a risk for bleeding.

### DRUGS FOR HEMORRHAGIC STROKE

**Calcium Channel Blockers:** After a subarachnoid hemorrhagic stroke, spasm of blood vessel occurs which closes off oxygen to the brain. Calcium causes contraction of blood vessels. While the calcium channel blockers relax the blood vessels. Time window for this drug is 6 hrs. It is not recommended for ischemic stroke eg. Nimidopine (Nimotop).

**Urokinase Irrigation:** Introducing irrigation tubes and administering urokinase (a thrombolytic agent) after surgically removing an aneurysm may help prevent spasm.

### Investigative Drugs Used to Protect or Restore Nerve Cells after a Stroke

#### Nerve-Protecting Agent: An upcoming urge to produce medications that may slow down or prevent the cascading process that destroys nerve cells after a stroke. Many drugs have been formulated that target the primary cascade of damage, eg glycine and glutamate that destroy the nerve cells after stroke. Other nerve protecting agents being investigated that have shown some promise include citicoline, clomethiazole, piracetam and ebselen.

### Agents for Nerve Regeneration: The main setback with the brain cells is that they are anti-mitotic. Scientists have observed neurogenesis in adult brain; this opens new vistas in neurological sciences. One investigative technique involves the transplanting of laboratory-grown nerve cells into the brains of stroke patients in order to improve motor and speech skills.

### Blood-Pressure Lowering Agents in Patients with or without Hypertension

Drug therapy is always recommended for people with hypertension, supplementation reduces the risk of stroke by 42%. ACE inhibitors may be particularly protective against stroke in many patients, including those with diabetes.

### Cholesterol Lowering Drugs

#### Statins: The cholesterol-managing HMG-CoA reductase inhibitors, commonly called statins, such as lovastatin (Mevacor), pravastatin (Pravachol) and simvastatin (Zocor), reduce the risk of a second stroke in people with high cholesterol levels and heart disease. These agents lower LDL (the so-called bad cholesterol) and raise HDL (the so-called good cholesterol) levels and help open up arteries. Recovery to stroke sufferers is effective.

#### Fibrates: These are agents used to improve cholesterol levels. They tend to increase HDL (the good cholesterol) and reduce levels of triglycerides (which are important health risk factors). Fibrates include gemfibrozil (Lopid), fenofibrate (Tricor) and bezafibrate.

### Hormone Replacement Therapy
The beneficial effects of estrogen on blood vessels that open blood flow to the heart should also serve the same purpose in the brain and protect against stroke.

Diet: A healthy diet rich in fruits and vegetables and low in salt and saturated fats may significantly lower the risk for a first ischemic stroke. Foods such as apples and tea, which are high in food chemicals called flavonoids, prove to be effective. Calcium, magnesium and potassium serve as electrolytes in the body, and may play a pivotal role in stroke. Oil and fat control play an important role in stroke control. Everyone should quit smoking and, if they drink alcohol at all, should do so in moderation. Caffeine drinkers, however, would do better to choose tea, which may have beneficial nutrients, and people with existing hypertension should avoid caffeine altogether (since caffeine may increase the risk for stroke in this group). The benefits of exercise on stroke are less established than on heart disease but recent studies showed fruitful results.

**Therapeutic strategies undertaken in the study for the salvage of compromised neurons**

Clinical trials involving various studies have increasingly targeted the evaluation of the effectiveness of various neuroprotectants. The results are sometimes encouraging but variable and thus confusing. Rationale for the possible clinical effectiveness of antioxidants and various other protectants in cerebral ischemia has arisen out of the many years of basic science generally illustrating the basic perpetrators of the disease in the processes involved. Using various mechanisms, neuroprotective agents save ischemic neurons from irreversible injury, 4 h after onset of ischemia damage occurs in the viable neurons. In humans the time window may be longer, but human patients tend to be older with comorbidities that may limit benefit. As neuroprotective drugs reduce ischemic damage this line of pharmaceutical research holds great promise. Drug selection included in the present study is as follows:

**A. UNANI FORMULATIONS**

- Khамиra Abreesham Vood Mastagiwala (KAUM)
- Majun Baladar (MB)
- Majun Khadar (MK)

**B. SYNTHETIC DRUGS**

- Deprenyl
- Resveratrol

**C. ELEMENTAL**

- Selenium (Sodium Selenite)

**A. GREEK MEDICINE (OR) TIBB-E-UNANI**

**Introduction**

Avicenna (Ibn Sina in Arabic, AD 980-1037), the ancient Unani physician with the most far reaching impact on the Islamic and the Western world, defined Unani medicine as the science by which we learn the various states of the body in health, and when not in health, and the means by which health is likely to be lost, is likely to be restored. Unani medicine, like any other form of medical science, strives to find the best possible ways by which a person can lead a healthy life with minimum sickness. It is different from other branches of medicine as the drugs it uses are natural in their sources and forms. It emphasizes on retaining natural compounds, which belong to the human's body and hence provide only natural remedies.

**Historical background**

Since the very beginning of life, there is death and disease, therefore the history of medicine is as old as medicine is as old as
the history of civilization. The study of medicine flourished in all ancient civilizations. Some ancient medical systems are Misri Tibb (Egyptian medicine), Hindi Tibb (Indian medicine), China Tibb (Chinese medicine) and Unani Tibb (Greek medicine). The Unani art of healing is still a living system and is practiced with a lot of zeal and fervor by Unani physicians, due to accumulation of pragmatic information over centuries. In the fifth century BC, Greek medicine was given form and recognition as a scientific system by Hippocrates or Buqarat (460-377 BC) who is considered the father of Unani medicine. Hippocrates freed medicine from the realm of superstition and magic. Hippocrates said: "Let your food be your medicine, and your medicine be your food". The Greek physician, Galen or Jalinoos (AD 131-210) was probably the most influential and prolific; he based his philosophy and theories on experimental evidence. Aristotle had said, "Nature does nothing without a purpose". Galen agreed and felt that since nature always had a clear purpose, an organ too must have a specific function. To affirm this, he defined the structure and functions of all body organs and established a concept of anatomy as well as physiology of the human body. Greeks were busy advancing medical knowledge in the West. Then came a time in the 13-14th century AD when Arab scholars introduced Unani system in India.

*Khameera Abreesham Uood Mastagiwala (KAUM)*

Herbs/minerals from plant and animal origin, forms the base of traditional Unani medicines (TUM) is almost ubiquitous. The dearth of scientific proofs has subdued the importance of this science, which was evolved ages ago. Healthcare professionals should be aware of its potential and researchers should strive to fill the numerous gaps overcoming this lack of communication. There is growing evidence for the efficacy of these herbal medicines (Ernst 2000). All TUM are mixtures of more than one active ingredient. In many cases it is uncertain which or how many constituents are pharmacologically important. On the other hand, the multitude of active ingredients obviously increases the likelihood of interactions. This concept, that a whole or partially purified extract of a plant offers advantages over a single isolated ingredient, also underpins the philosophy of Unani medicine. Evidence to support the occurrence of synergy in traditional medicines is now accumulating.

*Khameera Abreesham Uood Mastagiwala (KAUM)*, a compound formulation used in the Indian System of Unani Medicine since ages, is extensively used in the treatment of arrhythmia, palpitation and cardiac debility, effectively stimulating the functioning of all the basic organs of the body (Kabeer 1951). Amongst the important ingredients of KAUM, Abreesham (Silk cocoon), Kehruba and Shahed (honey) are only considered.

**ABRESHAM (silk cocoon)**

Silk is an animal fibre, produced by caterpillars belonging to the genus *Bombyx*. The filament is bonded by secretion of sericin that is a major cocoon protein (Voegeli et al. 1993). Usually the sericin content of the cocoon shell is at the maximum level at the outside layer 1 becomes progressively lower at the middle layers 2 and 3 and the absolute minimum at the inside layer 4 as shown in Table A.
Antioxidant properties of sericin have been described by many workers (Zhaorigetu et al. 2001, Kato et al. 1998). Its usefulness as a chemo-preventive agent for colon carcinogenesis is also reported (Kato et al. 2000). Tamada et al. (2004) reported the anticoagulant activity of sulphated sericin, which adds to its therapeutic potential. Silk is carminative, diaphoretic, and emmenagogue and used as a diaphoretic for children and for chronic hemorrhage of the uterus in adults (Kabeer 1951). These properties are of prime importance because it enhances the oxygen diffusivity thereby increasing alveolar oxygen transport and enhances pulmonary oxygenation. It improves cerebral oxygenation in hemorrhaged rats and positively acts in the atherosclerosis and arthritis treatment.

VALERIA

Among the various species of genus Valeriana Linn, many are of therapeutic importance. One among them is Valeriana officinalis Linn (Nardostachys jatamansi (NJ) or Sumbul teeb a constituent of Khumeera Ahreesbam Uood Mastaghmk (NJ) or Sumbul teeb a constituent of Khumeera Ahreesbam Uood Mastaghmk whose individual antioxidant potential against cerebral ischemia was reported by Salim et al. (2003). Valeriana species have been prescribed as a remedy for hysteria, hypochondriasis, nervous and emotional troubles and their sedative and tranquillising property are also highlighted. Malva et al. (2004) indicated the neuroprotective property of V. officinalis against signaling pathways involving Ca"+ (i) and the redox state of the cells, which can be of immense help in neurodegenerative disorders. Progressive improvement in attention, immediate recall, intelligence and visuospatial functions after drug therapy were seen. Combinatorial effects of kava and valerian, was found to be beneficial to health as it reduces physiological reactivity during stressful situations (Cropley et al. 2002).

HONEY

Health and Therapeutic Attributes Of Honey

Honey, a commonest sweetener throughout the world from ancient times, has many health-promoting and curative properties. About 6000 BC or say 8000 years back the medicinal aspects of honey were documented.

Antioxidant capacity of honey is the combined activity of a wide range of compounds including phenolics, peptides, organic acids, enzymes, Maillard reaction products, and possibly other minor components. The phenolic compounds contribute significantly to the antioxidant

Table A. Sericin content to different layers of cocoon shell

<table>
<thead>
<tr>
<th>Cocoon layers</th>
<th>Race A (%)</th>
<th>Race B (%)</th>
<th>Race C (%)</th>
<th>Race D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside</td>
<td>31.40</td>
<td>32.08</td>
<td>34.13</td>
<td>33.15</td>
</tr>
<tr>
<td>1</td>
<td>23.45</td>
<td>29.29</td>
<td>27.50</td>
<td>27.71</td>
</tr>
<tr>
<td>2</td>
<td>20.11</td>
<td>22.22</td>
<td>23.96</td>
<td>23.47</td>
</tr>
<tr>
<td>3</td>
<td>18.12</td>
<td>20.63</td>
<td>21.54</td>
<td>21.33</td>
</tr>
<tr>
<td>Inside</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
capacity of honey but are not solely responsible for it. Honey contains a ‘sucralfate-like’ substance, affords protection against ischemia-reperfusion-induced injuries in the rat stomach and gastric protection (Ali 1995, Schramm et al. 2003). It has a bacteriostatic, bactericidal and anti-microbial (Mural et al. 2004). The therapeutic potential of honey, which contains about 181 substances (White 1979), is gradually growing and scientific evidences for the effectiveness of honey in several experimental and clinical conditions are emerging (Al-Mamary et al. 2002). The medicinal properties of honey have been well described for a variety of medicinal and nutritional purposes (Cherbuliez 2001, Molan 2001, Cherbuliez and Domerego 2003, Snow and Manley-Harris, 2004). The therapeutic intake of honey for treating gastroenteric disorders (29.2%), respiratory infections (21%), treatment of oral diseases, treating peptic ulcers, gastritis, oral surgery is well documented (Cherbuliez 2001, Cherbuliez and Domerego 2003).

**Majun Baladar (MB)**

Avicenna (Ibn Sina in Arabic, AD 980-1037) reported the pertinent qualities of Majun Baladar (MB) in his prized book followed by Kabeer 1951. However, the neuroprotective effect of Majun Baladar (MB) has not been studied in focal cerebral ischemia. The 21 constituents of MB are pharmacologically active individually as well as in synergism. Among its 21 constituents, sesame, almond, saffron, honey, ginger are components that are commonly consumed in diet.

**SESAME**

Egyptians used sesame seed as medicine as early as 1500 B.C. Sesame is an important oilseed crop of the world, a highly stable oil and nutritious protein and meal. Sesame seed contains 25% protein. It is also an ingredient in Ayurvedic oils under the Indian System of Medicine (Suja et al. 2004). Sesame lignans include sesamin and sesaminol that have various physiological effects being antioxidant (Yamashita et al. 1992, Kang et al. 1998, Akimoto et al. 1993). Anticarcinogen, blood pressure lowering and serum lipid lowering activities in experimental animals and humans would potentiate the characteristics of sesame in improving human health (Hirose et al. 1992, Marumura et al. 1995, 1998, Hirata et al. 1996, Ashakumary et al. 1999). Sesame is reported to possess antiaging properties, alleviation of symptoms of alcohol withdrawal etc. (Hirata et al. 1996). Recently, investigation on natural antioxidants from industrial by products revealed the possibility of developing an antioxidant extract from sesame cake, (Patent proposal, U.S. Appl. 60/404.004, Suja et al. 2004).

**SAFFRON (Crocus sativus L)**

Saffron-colored compounds are crocins, which are unusual water-soluble carotenoids (mono and diglycosyl esters of a polyene dicarboxylic acid, named crocetin). The digentiobiosyl ester of crocetin - α-crocin is the major component of saffron. Saffron contains proteins, sugars, vitamins, flavonoids, amino acids, mineral matter, gums, and other chemical compounds (Abdullaev 1993, Rios et al. 1996, Winterhalter and Straubinger 2000). Saffron increased the intracellular levels of reduced glutathione and glutathione-related enzymes suggested a possible antioxidant activity of saffron (Nair et al. 1992, Abdullaev et al. 1995, 1996). Saffron extract and its purified characteristic compounds crocin, safranal, picrocrocin, and β-carotene inhibit different types of tumor cell growth. Antitumor action of saffron is because of its inhibitory effect on free radical chain reactions, as most carotenoids are lipid-soluble and act as membrane-associated high-efficiency free-radical scavengers, which is connected with...
Review of Literature

their antioxidant properties. Saffron extract is non-toxic and non-mutagenic. Treatment of tumor cells with saffron resulted in an increase in the level of intracellular sulphhydryl compounds, and all these factors concomitantly explain the antioxidant activity of saffron (Giaccio 2004).

ALMOND (Prunus)

Prunes, good energy source in the form of simple sugars. Serving 100 g fulfills the daily requirement for boron (2-3 mg), potassium (745 mg/100g) that is postulated to play a role in prevention of osteoporosis and cardiovascular health reducing cardiovascular diseases. Polyphenolics present in nuts, especially their skin, contribute to their cardioprotective action, their bioavailability has positive effects in protecting GSH and LDL from oxidations in healthy older adults (Chen et al. 2004). Dried prunes contain approximately 6.1 g of dietary fibre/100g and high sorbitol content (14.7 and 6.1g/100g in dried prune and prune juice respectively (Kikuzaki et al. 2004) that maintain a rapid rise in blood sugar concentration, possibly because of high fiber, fructose, and sorbitol content. Prunes contain large amounts of phenolic compounds (184 mg/100 g), mainly as neochlorogenic and chlorogenic acids, which may aid in the laxative action and delay glucose absorption. Prunes have shown high antioxidant activity on the basis of the oxygen radical absorbance capacity (ORAC), and their major antioxidant components are caffeoylquinic acid isomers (Kayano et al. 2003). Ethanol extract of prune led to isolation of a novel compound, 4-amino-4-carboxychroman-2-one, together with four known compounds (p-coumaric acid, vanillic acid beta-glucoside, protocatechuic acid, and caffeic acid), structures with remarkable antioxidant properties (Kayano et al. 2003, Takeoka and Dao 2003). Almond hull extracts showed increased antioxidant activity thereby suggesting their natural potential role in dietary antioxidants (Takeoka and Dao 2003). These dietary plant sterols have been demonstrated to reduce serum cholesterol levels and also may inhibit colon cancer development, composition of almond hulls are identified as chlorogenic acid, cryptochlorogenic acid and neochlorogenic acid, which exhibit antioxidant and anticarcinogenic properties (Takeoka and Dao 2002).

GINGER (Zingiber officinale)

From the Sanskrit word ‘Shringavera’ (meaning ‘horn body’) the Latin name ‘Zingiber’ was derived for ginger. History of ginger dates back to more than 5000 years ago the ancient Chinese and Indians looked upon it as a “universal medicine”. Ginger is the active ingredient of >50% traditional formulations with myriad of health ailments treated with it. The active components, gingerol and paradol are potent antioxidants in also has high content of iron and calcium, an anti-inflammatory compound. Ginger is called as nature’s aspirin because it helps to clear the build up in clogged arteries because of its blood thinning properties, it also strengthens cardiac muscles and lowers serum cholesterol levels. The powdered rhizome of Zingiber officinale has been found to be more effective than dimenhydrinate (Dramamine) in reducing motion sickness in individuals highly susceptible to this malady” (Lancet, 1982). Aids digestion by increasing the digestive movements and stimulating enzymes for digestion. Anti-inflammatory action was proved by its potential in arthritis treatment, relief from pain and swelling. Zinaxin, a drug launched in UK for the treatment of arthritis was formulated on ginger as a main ingredient. Effective against tumor growth, migraines,
rheumatism, anti-bacterial, anti-ulcer activity. The International Journal of Obesity reported in October 1992 that ginger burns calories and so aids weight loss.

One more important constituent in which the formulation is prepared is honey, which we have already discussed in KAUM.

MAJUN'KHAĐAR

Majun Khadar (MK) is renowned traditional Unani formulation, which has been used since ages for the prophylaxis of various disorders. It has 28 components, which act synergistically against myriad diseases. It has got many vital components that are individually proven for their efficacy. Borage, cinnamon, honey and saffron are considered here.

BORAGE

(Borago officinalis L)

The fatty oil, obtained from borage seeds ("borage oil", "starflower oil") is rich in polysaturated fatty acids, e.g., linolenic acid (20%). Antioxidant activity of crude borage extract was determined using DPPH free radical method, a rapid decrease in absorbance and a very high hydrogen donating capacity towards 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical was found. The antioxidant components were separated using HPLC. The HPLC analysis of borage extract revealed the presence of several radical scavenging components in the borage extract with high radical quenching ability. The dominant antioxidant compound in the crude extract of borage leaves was identified as rosmarinic acid (Bandoniene and Markovic 2002).

CINNAMON (Cinnamomum zeylanicum)

The essential oil of cinnamon bark is dominated by the two phenylpropanoids cinnamaldheyde (3-phenyl-acrolein, 65-75%) and eugenol (4-(1-propene-3-yl)-2-methoxy-phenol, 5 to 10%). Other phenylpropanoids (safole, coumarine (0.6%) cinnamic acid esters), mono- and sesquiterpenes, although occurring only in traces, do significantly influence the taste of cinnamon. Classic Chinese Formula, Gui Zhi Fu Ling Wan /Cinnamon and Hoeken Formula, is known to disperse stagnant blood and lumps in the lower abdomen, manifesting as a variety of urogenital disorders because of the ability of cinnamon twig to work as a vasodilator and promoter of blood flow (Elixir Tonics and Teas 1999). Treatment of diabetes with cinnamon dates back to approximately 1550 B.C. Specific antioxidant phytochemicals that have been identified in cinnamon include epicatechin, camphene, eugenol, gamma-terpinene, phenol, salicylic acid, and tannins. Cinnamon, which is usually high in flavonoids, also may be synergistic with vitamins and trace minerals (Darren et al. 2004).

One more important constituent in which the formulation is prepared is honey, and saffron is already discussed.

B. SYNTHETIC DRUGS

Resveratrol

Polyphenolic phytochemicals are compounds synthesized by plants (mainly spermatophytes), such as flavanoids, proanthocyanadin, phenolic acids the latter are of intense interest (Choi et al. 2003). They are integral part of human and animal diets among them resveratrol (3,4',5 trihydroxystilbene) is point of interest, which is present in mulberries, peanuts, grape berry skins and seeds but not in the flesh which has been identified as the major active compound of stilbene phytoalexins.
(Fremont et al. 1999, 2000, Ignatowicz and Baer-Dubowska 2001). It is synthesized by Polygonum cuspidatum (Kojokan in Japanese) roots belonging to the family Polygonaceae. Detected in grapevines (Vitis vinifera) in 1976 by Lancake and Pryce who found that the compound was synthesized by leaf tissues in response to fungal infection or exposure to ultraviolet light. Resveratrol came into light with its presence observed by Siemann and Creasy (1992).

Properties of red wine are under investigations (Figure 14). Polyphenolics occur primarily in conjugated form with glucose as the most common sugar residue.

French Paradox

Resveratrol is attracting great interest in science due to the "French paradox" which states that despite high fat intake, mortality from coronary heart disease is lower in some regions of France than in the other developed countries due to the regular wine consumption. In the early 1990's television reports on the French Paradox noted that while smoking and fat intake in France are higher than in United States, the incidence of myocardial infarction is one-third that of United States (Renaud and De Lorgeril, 1992). Various epidemiological studies have shown an inverse correlation between the level of intake of dietary flavonoids and death from coronary artery disease (CAD). In a larger group of 16 Cohorts in seven countries, flavonoid intake was inversely related to CAD mortality.

Characterization And Analysis

Resveratrol (3,4', 5 trihydroxystilbene) exists in two forms i.e. cis and trans. The cis isomer was never identified in grape extracts but is present in other foods.

It is a parent molecule of a family of polymers namely viniferins, the extraction from natural sources is a time consuming and yields low amounts of the compound.

Trials have shown that it was stable for several months (except in high pH buffers) when completely protected from light (Trela and Waterhouse 1996). Analyses require the complete extraction within a short period of time to reduce denaturation and isomerization. A number of methods have developed like high performance liquid chromatography (HPLC) and gas chromatography (GC) coupled or not with mass spectrometric (MS) detection. Goldberg et al. (1996) proposed a rapid and sensitive means of detection in grape juices or jams with GC-MS method. Electrophoresis was also used for the analysis of two isomers of resveratrol.

Kinetics And Bioavailability

The response of living organisms to a substance depends on the bioavailability of the compound. Human trials were conducted to evaluate the absorption of resveratrol, which was observed by the intestines. Resveratrol is absorbed quickly in the blood stream and significant concentrations in the blood and the plasma were detected (Bertelli et al. 1996). Later the same group in 1998 showed the rapid transport to the blood within 1 h after administration. The levels were declined rapidly with an apparent half-life of 4h (Wang et al. 2002). Resveratrol is present in the glucuronide form, suggesting that the compound is transported and modified by the hepatic system. The plasma kinetics and
bioavailability by an intragastric tube (28μg/rat) demonstrated the pharmacokinetics by one or two-compartment model, a significant bioavailability and a strong affinity for the liver and kidney were observed (Bertelli et al. 1996). Workers suggest that in long term, an average drinker of wine can absorb sufficient quantity of resveratrol to explain the beneficial effect of red wine on human health. Wang et al. (2002) reported the golden property of resveratrol by demonstrating its ability to cross the blood-brain barrier (BBB) and incorporate into brain tissues. Serum resveratrol levels were reported to be highest at 1 h and were declined by 7.5% by 24 h. On the other hand, resveratrol in the liver and brain reached a peak at 4h, followed by a steady decline with time (Wang et al. 2002).

Presence In Red Wine/Peanuts

An 8-ounce glass of red wine provides approximately 640 mg of resveratrol, while a handful of peanuts provide about 73 mg of resveratrol. Resveratrol supplements are generally taken in the amount of 200–600 mg/day. The optimal level of intake is not known. Due to the risks involved with drinking alcohol, drinking red wine cannot be recommended as a means of preventing heart disease until more information is known.

Pharmacological Interventions

1. Vasorelaxing And Anti-platelet Aggregation Activity

In vitro and in vivo animal studies reported that resveratrol decreases the “stickiness” of blood platelets and helps blood vessels remain open and flexible (Bertelli et al. 1996, Chen and Pace-Asciak 1996). Platelets play a major role in atherosclerotic diseases and reduction of platelet activity by medications reduces the incidence and severity of disease. Red wine and grapes contain polyphenolic compounds, including flavonoids, which can reduce platelet aggregation and have been associated with lower rates of cardiovascular disease with its known vasodilator actions (Keevil et al. 2000, Bhat et al. 2001, Celestini et al. 2003). Antiplatelet aggregation activity (Chung et al. 1992) as well as coronary vasodilator action (Inamori et al. 1987) anti-leukemic, (Mannila et al. 1993) antifungal, (Langeake et al. 1979) and protein-tyrosine kinase inhibitory action (Jayatilake et al. 1993) of resveratrol is known. Since these compounds are available in small amounts from natural sources, the synthesis of resveratrol and related hydroxystilbenes and their glucosides has been undertaken to provide larger quantities for further biological evaluation (Orsini et al. 1997). It might provide protection as: (1) inhibition of the oxidation of low-density lipoprotein cholesterol an effect which is expected to inhibit atherosclerotic changes, (2) attenuation of platelet aggregation by inhibition of the metabolism of arachidonic acid. Resveratrol modulates platelet coagulation through multiple mechanisms. It inhibited platelet adhesion to type I collagen, which is the first step of platelet activation, it also reduced platelet aggregation induced by thrombin (Ignatowicz and Baer-Dubowska 2001). The vasorelaxative activity of resveratrol depends also on direct stimulation of K_Ca channels in endothelial cells (Li et al. 2000). After the exposure of resveratrol in the studies of calcium channels in endothelial cells has demonstrated the vasorelaxant activity mediated by nitric oxide, the vasodilation upon resveratrol treatment was not reversed by the NOS inhibitor (Chen and Pace-Asciak 1996). It has also been suggested that resveratrol blocks the in vitro aggregation due to the inhibition of mitogen activated protein (MAP) kinases in platelets (Kirk et al. 2000). The reduction of Tissue factor (TF) expression in vascular cells may also contribute to the anti-aggregatory effect of resveratrol (Pendurthi et al. 1999).
2. Cardiovascular And Cerebrovascular Effects

Resveratrol protects the cardiovascular system by mechanisms that include defense against ischemic-reperfusion injury, promotion of vasorelaxation, protection and maintenance of intact endothelium, anti-atherosclerotic properties, inhibition of low-density lipoprotein oxidation, suppression of platelet aggregation, and estrogen-like actions. (Pace-Asciak et al. 1996, Keevil et al. 2000, Hao and He 2004). The Daily Times, 2005 reported that moderate alcohol consumption could apparently reduce the risk of clots because of its favorable effect on lipoproteins, during ischemic strokes. The platelet inhibitory effect of the flavonoids in grape juice may decrease the risk of heart blockage and heart attack.

The debate arising from these studies evaluated that alcohol may reduce risk by increasing HDL concentration (Gaziano et al. 1993), while the non-alcoholic constituents in red and grapes have protective antioxidant and antiplatelet properties (Demrow et al. 1995). Resveratrol interfered with a number of cellular circuitries, which led to the diminishment of the atherogenic changes in plasma and artery wall and improving the outcome after ischemia/reperfusion injury, by preventing lipids from peroxidative degradation and discontinued uptake of oxy-LDL in the vascular wall in a concentration-dependent manner. Liver parenchymal cells in culture, treated with resveratrol, showed reduced secretion of esterified cholesterol and triglycerides although the intracellular triglyceride level was unchanged. It allows the supposition that resveratrol reduces the secretion of VLDL from the liver, which is transformed into LDL in blood circulation, thus blocking hepatic lipoprotein metabolism. Resveratrol may protect LDL molecules against peroxidation through antioxidative activity and metal.

3. Antioxidant Activity

Polyphenolic compounds represent potent antioxidants (Rice-Evans et al. 1995). Fremont (2000) demonstrated that it is not only an antioxidant, antimutagen but could also reduce cell death. Amphipathic character, allows the protection of cellular and sub-cellular components. The probable mechanisms are free radical scavenging and selective interference with a multitude of factors affecting the division cycle of rapidly and abnormally proliferating mammalian cells. Because grape extracts are a convenient alimentary source of salutary phytochemicals to supplement currently prevalent occidental food and resveratrol appears to be especially useful. It could conveniently be added in biosignificant amounts to the grape extracts provided that their extraction, contents, and quality controls are instituted (Bertelli et al. 1996, Chen and Pace-Asciak, 1996). Its antioxidative properties are widely reported (Fremont 2000, Wang et al. 2004). The flavonoids are also known to inhibit cyclooxygenases and phosphodiesterase enzymes.

Study by Virgili and Contestabile 2000 demonstrated that chronic administration of Resveratrol to young adult rats could significantly decrease neuronal damage caused by systemic injection of kainic acid, reduction in the infarct size was also observed (Huang et al. 2001). Suppressed mitochondria-induced production of ROS (Zini et al. 1999). Resveratrol has been proved to scavenge peroxyl and hydroxyl radicals in reperfused postischemic isolated rat hearts, to limit infarct size and to reduce the formation of malondialdehyde, a non-specific marker of lipid peroxidation occurring under oxidative stress (Sato et al. 2000). Zini et al. (1999) recognized the antioxidant properties through three different mechanisms through which this phytalexin exerts its action:

- Resveratrol is supposed to compete with coenzyme Q and it decreases the
Review of literature

oxidative chain complex III, which are the site of ROS generation (Zini et al. 1999).

- Scavenges superoxide radicals formed in the mitochondria and inhibits lipid peroxidation induced by Fenton reaction products (Zini et al. 1999).

- Normalization of myeloperoxidase and oxidized- -glutathione reductase activities upon resveratrol treatment (Jang and Pezzuto, 1999).

- Inhibition of monoamine oxidase A (MAO-A) may be regarded as a prime factor responsible for its antioxidant activity. Resveratrol inhibited brain monoamine oxidase in rats (IC50 of 2μM), even though it was lacking the structural features of classic monoamine oxidase inhibitors.

4. Apoptosis

Emerging literature points to the low intestinal absorbance of polyphenols, suggest that the ability of polyphenols and their in vivo metabolites to interact with cell signaling cascades such as apoptosis and redox-sensitive cell-signaling pathways that may be a major mechanism of action (Jang et al. 1997, Schroeter et al. 2001). Resveratrol showed protection against oxidative DNA damage in stroke-prone hypertensive rats (Mizutani 2001). Inhibition of apoptotic cell death induced by oxidative stress was also reported (Sun et al. 1998, 2001).

5. Estrogenic Activity

Neuroprotective effects which salvage cells from ischemic pathways of estrogens are well documented. Estrogen is studied well in animal /cell injury models of cerebral ischemia (Hurn and Brass 2003, Wise 2003) with nearly favorable results i.e. cell salvage from ischemic death pathways. Both chronic and acute pretreatment of estrogen ameliorated ischemic damage in focal cerebral ischemia (Yang et al. 2000). Estradiol affects neuronal excitability (Brawer et al. 1983), hippocampus (Gu et al. 1999) and nucleus accumbens (Gibbs et al. 1997). Variety of neurotoxic stimuli including oxidative stress, excitotoxic insults and β-amyloid toxicity induce significant neuronal cell death that can be attenuated by estrogenic compounds (Behl et al. 1998, Wise et al. 2001). The structural similarity of resveratrol to diethylstilbestrol, a synthetic estrogen, has led to the hypothesis that it might express a phytoestrogenic function. Endogenous estrogens have known cardioprotective properties and it seems very likely that phytoestrogens present in red wine could exert similar action (Gehm et al. 1997).

Other Effects

Besides the ability to inhibit delayed neuronal damage, resveratrol showed a substantial decrease in glial cell activation due to the ischemic insult (Wang et al. 2003). Inhibition of cytokine induction of inducible nitric oxide synthetase and secretory phospholipase A2 is reported (Li and Sun 1998). In human polymorphonuclear neutrophils, resveratrol decreased the amount of lipooxygenase proinflammatory products (5-hydroxyeicosatetraenoic acid, 5,12-dihydroxyeicosatetraenic and leukotriene C4) (Kimura et al. 1995), inhibited the lysosomal enzymes release upon calcium ionophore exposure, and decreased ROS generation (Jang et al. 1999). Suppression of phospholipase A and COX activities, along with inhibition of phospho-diesterase leading to an increase in the amount of cyclic nucleotide and inhibition of protein kinases involved in cell signaling (Soleas et al. 1997) added to the cardioprotective and anti-inflammatory properties of resveratrol.

Deprenyl

Synonyms of Deprenyl
The other names by which deprenyl is known Selegiline, Jamex, Eldepryl, and Movergan.

Pharmacological Glimpses

Irreversible inhibitor of monoamine oxidase (MAO) B, discovered in 1962 branded as the "golden standard" of MAO research. Monoamine oxidase (MAO) is an enzyme, present in most tissues, especially in the liver and the nervous system that oxidizes various physiologically and pathologically important monoamine neurotransmitters and hormones such as dopamine, noradrenaline, adrenaline, and serotonin.

Two types of MAO, i.e. type A (MAO-A) and type B (MAO-B) were first discovered pharmacologically. MAO-A is inhibited by clorgyline and MAO-B, by deprenyl. MAO-A and MAO-B are made of similar but different polypeptides and encoded by different nuclear genes located on the X chromosome. MAO-A and MAO-B genes consist of 15 exons with identical intron-exon organization, suggesting that they were derived from a common ancestral gene. Both enzymes require a flavin cofactor, flavin adenine dinucleotide (FAD), which binds to the cysteine residue of a pentapeptide sequence (Ser-Gly-Gly-Cys-Tyr). Both enzymes exist on the outer membrane of mitochondria of various types of cells in various tissues including the brain. MAO-A oxidizes noradrenaline and serotonin; and MAO-B, mainly beta-phenylethylamine. In the human brain, MAO-A exists in catecholaminergic neurons, but MAO-B is found in serotonergic neurons and glial cells. MAO-A knockout mice exhibit increased serotonin levels and aggressive behavior, whereas MAO-B knockout mice show little behavioral change. The gene knockout mice of MAO-A or MAO-B, together with the observation that some humans lack MAO-A, MAO-B, or both have contributed to our understanding of the function of MAO-A and MAO-B in health and disease. MAO-A and MAO-B may be closely related to various neuropsychiatric disorders such as depression and Parkinson's disease, and inhibitors of them are the subjects of drug development for such diseases.

Deprenyl is a propargylamine derivative, mechanisms underlying the ameliorating actions of deprenyl in Parkinson's is not so far clear. It is suggested that resulting elevation of specific substrate PEA may be helpful in some respect (Birkmeyer et al. 1977). PEA acts as a dopaminergic neuromodulator (Paterson et al. 1990), which might be suggestive of the important factors of deprenyl. It is not apparent from the studies the beneficial effects of deprenyl may result from elevation of the levels of dopamine or PEA alone or may be both of them are necessary. PEA interacts with a receptor site in brain (Hauger et al. 1982). It is not yet confirmed and may be due to the results of association of PEA with MAO-B. In vitro/in vivo studies have shown PEA to inhibit uptake and release of amine neurotransmitters, including dopamine (Baker et al. 1976). PEA is not a classical neurotransmitter; it is not released through vesicular exocytosis, but through simple diffusion. It may effect by potentiating the post-synaptic effects of dopamine (Paterson et al. 1990). MAO-B is not located intraneuronally in the striatum (O'Carroll et al. 1987), therefore pharmacological action of PEA occurs extraneuronally (Youdim and Tipton 2002). MAO-B prefers b-phenylethylamine as a substrate, and is inactivated by deprenyl (Knoll 1978) as a selective inhibitor.

Cheese Effect

Monoamine oxidase inhibitors strongly potentiate the catecholamine releasing effect
Review of Literature

The Mechanism of "Cheese" Reaction

Figure 15: Showing the "cheese effect".

of tyramine whereas deprenyl inhibits it and is free from the "Cheese effect" which makes it safe for therapeutic treatment. In late 1950's and 1960's, iproniazid and other MAO inhibitors were known for their anti-depressant activity. But their therapeutic efficacy was seriously limited because of liver toxicity and by what became known as the "cheese reaction" which was due to the presence of tyramine in many fermented foods including cheese that was not deaminated by MAO in the intestine of patients treated with these MAO-inhibitors. These side effects virtually arrested the development and assessment of other MAO-inhibitors as antidepressants. Hepatotoxicity was associated with hydrazine derived drugs which was not there with other non-hydrazine MAO inhibitors, therefore "cheese effect" was the major set back for these effective antidepressants (Figure 15). Tyramine and other indirectly acting sympathomimetic amines present in food and beverages are metabolized by MAO to inactive substances. If peripheral including intestinal MAO are inhibited these amines then gain access to the circulatory system and result in the significant release of noradrenaline from sympathetic neurons. This can result in a severe hypertensive reaction. MAO exists in two forms in brain and in most tissues of animals and humans. MAO, Type-A and Type-B, MAO-A type of enzyme was inhibited selectively by clorgyline, was responsible for oxidative deamination of noradrenaline and serotonin. By contrast, MAO-B was resistant to inhibition by clorgyline and preferentially metabolized benzylamine and phenylethylamine. Tyramine and dopamine were metabolized equally well by both forms of the enzyme (O'Carroll et al. 1987, Youdim and Tipton 2002).

MAO-B accounts for about 80% of total MAO activity in human basal ganglia mainly localized in glial cells and in a few specific neuronal cells, e.g. raphe nuclei. MAO-A is present in the extraneuronal compartment and within the dopaminergic; serotonergic and noradrenergic nerve terminals, where it is involved in the intraneuronal metabolism of these amines.

Deprenyl and Parkinsonism

Deprenyl is used in the treatment of Parkinson's disease. Approximately one-half of Parkinsonian patients develop depression requiring antidepressant drug treatment (Ritter and Alexander 1997). This is where deprenyl's, potent antidepressant action comes into play. Due to its dopamine potentiating capacity, it became a registered drug in the treatment of Parkinson's disease with a wide range of pharmacological activities. All of them are not related to its MAO-B inhibitory potency. Beside its dopamine potentiating effect, it renders protection against a number of dopaminergic, cholinergic and noradrenergic neurotoxins with a complex mechanism of action (Magyar and Szende 2004). It lacks catecholamine-releasing activity; it is devoid of amphetamine like dependence capacity. In PD it has been shown to postpone the need for levodopa and sparing levodopa in early PD. It is also useful in the management of end-of-dose akinesia in the fully developed disease (Birkmayer et al. 1977).

Deprenyl and Oxidative stress

Up regulation of activities of antioxidant enzymes such as superoxide dismutase
(SOD) and catalase (CAT) may be causally related to the effect of deprenyl on animal survival primarily because of the parallelism of the dose–response efficacies of deprenyl on these two seemingly different effects of the drug (Kitani et al. 1999, 2002). Deprenyl enhances SOD and CAT activity in the striatum and protects nigrostriatal dopaminergic neurons from selective neurotoxins (6-Hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1, 2,3,6-tetrahydro-pyridine (MPTP), noradrenergic (DSP-4), and cholinergic neurotransmitters (AF64A). Deprenyl showed significant protection on behavior (memory acquisition and memory retention) and against thiobarbituric acid reactive substances after global cerebral ischemia suggesting its antioxidant status (Maia et al. 2004). Inducing antioxidant enzymes and decreasing the formation of ROS, deprenyl was able to combat an oxidative challenge implicated as a common causative factor in neurodegenerative diseases. Deprenyl offers protection against DNA damage and oxidative stress by hydroxyl and peroxyl radical trapping and against excitotoxic damage from glutamate with stimulating the release of SOD. Clinical benefits of deprenyl via different mechanisms in neurodegenerative diseases are well documented (Fatton 1998). Its proposed action while protecting the neurons from oxidative stress may be due the ability of reducing the production of H$_2$O$_2$ (Cohen and Spina 1989).

Deprnely and Apoprotosis

Deprenyl has neuroprotective, trophic like neurorescue and apoptosis reducing properties (Knoll et al. 1978, 1999, Maruyama and Naoi 1999). Deprenyl interferes with early apoptotic signalling events induced by various kinds of insults in cell cultures of neuroectodermal origin, thus protecting cells from apoptotic death. Deprenyl requires metabolic conversion to an unidentified metabolite to exert its antiapoptotic effect, which serves to protect the integrity of the mitochondrion by inducing transcriptional and translational changes (Magyar and Szende 2004). Effect of deprenyl on 3,4-Methylenedioxymethamphetamine (MDMA)-induced serotonergic neurotoxicity was assessed in the striatum, hippocampus and frontal cortex of rats reported in blockage of malondialdehyde formation. Tryptophan hydroxylase (TPH) activity was also significantly reduced 18 hr after MDMA (Sprague and Nichols 1995). Deprenyl and its analogues are neuroprotective to dopamine neurons, protects the cells from apoptosis in a dose-dependent way even after it was washed out, suggesting that it may initiate the intracellular process to repress the apoptotic death program (Magyar and Szende 2004). Structure-activity relationship of (-) deprenyl analogues revealed that a N-propargyl residue with adequate size of hydrophobic structure is essentially required for the anti-apoptotic activity. Therefore, it can be regarded as an anti-apoptotic agent, which causes delay in the deterioration of neurons during advancing ageing and in neurodegenerative disorders (Maruyama and Naoi 1999, Simon et al. 2001). Magyar and Szende (2004) reported that 100, or

Neuro-Humoral Events And Mobilisation Induced By Deprenyl

Upregulation of antioxidant enzymes led to an increased live span of the animals (Kitani et al. 1999, 2000). Deprenyl is also able to mobilize many humoral factors, which include neurotrophic factors (Semikova et al. 1996) and other interleukins such as IL-2, IL-6 etc (ThyagaRajan et al. 2002). Anti-tumurogenic and anti-immunomodulatory effects have shown that deprenyl enhances natural killer (NK) cell activities and restores both neuro-adrenergic innervation and norepinephrine concentration in the spleen which in turn effect the survival in animals (ThyagaRajan and Felt'en 2002, ThyagaRajan et al. 1998, 2000).
Review of literature

even 1000 times lower dose of (-)-deprenyl can be offered in human therapy to protect, or slow down neuronal degeneration, than what is used presently. With low dose of the drug the dopaminergic adverse events could be avoided, while anti-apoptotic activity might be preserved (Magyar and Szende 2004). In some degenerative retinal diseases, ischemia plays an important role in neuronal injury where neurotrophic factor deprivation and hypoxia are the major factors responsible for neuronal injury. Apoptosis induced by these assaults was blocked partially on the retinal neurons by 1-deprenyl suggesting its anti-apoptotic effects. Decreased DNA fragmentation and positively stained apoptotic cells, increased cell survival under neurotrophic factor deprivation and hypoxia were also reported as a result of deprenyl treatment (Xu et al. 1999). In PC12 cell culture system, up regulation of Bcl-2 and reduction in the number of apoptotic cells that resulted in maintained Bcl-2 expression thereby keeping the mitochondria functionally intact. Thus, finally ensuing the activation of the apoptotic execution machinery was reported with deprenyl treatments (Tatton et al. 1994, Kluck 1997).

Deprenyl and Mitochondria

Mitochondrion is also protected by deprenyl via its effects on mitochondrial membrane permeability. It directly interacts with the pore-forming structures. If the mitochondrial theory of aging is correct, then the root cause of aging is damage to mitochondrial DNA by free radical leakage from adjacent respiratory proteins. Deprenyl itself is not an elixir of eternal youth. But its current "off-label" use by life-extensionists prefigures the longevity-enhancing mitochondrial medicine of decades to come. Neuroprotection offered by its metabolites, like (-)-desmethyl deprenyl, (-)- and (+)-methyl amphetamine was suggested (Tatton et al. 1996). With the reported stabilisation of the mitochondrial membrane and influencing the balance between the bcl2 and bax genes importance of the protective mechanism of action of (-)-deprenyl came to light in a different side, which was not elucidated earlier (Tatton, 1998, Marchi et al. 2003).

Deprenyl and Animal Behavior

Aging leads to memory impairments. Oxidative damage plays its role in aging process including cognitive deficits, age-related impairments in spatial memory and learning, which is alleviated by antioxidant treatment. Deprenyl afforded to be potent antioxidant by improving the antioxidant enzyme status and reducing lipid peroxidation (Kiray et al. 2004). Deprenyl tends to extend the life expectancy of rats by some 20%, enhances libido and endurance and independently improves cognitive performance in Alzheimers patients and in some healthy normal. It is used successfully to treat canine cognitive dysfunction syndrome (CDS) in dogs. Deprenyl improve the learning performance of aged rats (Knoll 1988).

Selenium

Named after Selene, the Greek goddess of the moon, selenium is an essential trace element, first discovered in 1817 by the Swedish chemist J J Berzelius. It is widely distributed throughout the earth’s crust in rocks, minerals, fossil fuels and volcanic material. Selenium is an integral part of many proteins, with catalytic and structural functions. Its nutritional deficiency leads to muscular dystrophy, endemic fatal cardiomyopathy (Keshan disease), chronic degenerative diseases in humans that could be prevented by selenium supplementation when used alone or in combination (Rayman 2002). Selenium is necessary for the full expression of selenium dependent enzymes (Li et al. 2001). Most important attribute of selenium is its presence in the body as selenocysteine in a number of proteins including the active site the enzyme glutathione peroxidase (GPx) (Zaffar et al.
2003, Ansari et al. 2005) and phospholipid hydroperoxide glutathione peroxidase (PHGPs) (Sunde 1994). eGPx and pGPx are involved in the regulation of intracellular hydrogen peroxide and lipid hydroperoxide concentrations (Weitzel and Wendel 1993). It is essential in biochemical and physiological processes, which include biosynthesis of coenzyme Q (a component of mitochondrial electron transport), regulation of ion fluxes across the membranes, maintenance of the integrity of keratins, stimulation of antibody synthesis, and activation of glutathione peroxidase (Figure 16).

Dietary Sources and Bioavailability of Selenium

Major dietary form of selenium is selenomethionine, which is wide spread in occurrence in plants and other foodstuffs. It is also present in liver, crabs and other shellfish. After ingestion, seleno-compounds are catabolised into an inorganic precursor before being incorporated as selenocysteine into selenoproteins. Brain is the last tissue affected by selenium deficiency whereas liver, kidney and lungs are the first.

Biochemistry of Selenium

More than thirty selenoproteins are identified, less than half of them are evaluated (Behne et al. 2000). Selenium exists in both organic as well as inorganic forms, which are utilized as nutrients. Inorganic selenium is reduced to selenide. Organic forms include selenocysteine and selenomethionine. Selenite is more effective than selenomethionine in preventing various pathological conditions. Antioxidant properties of some selenoproteins, glutathione peroxidase is important in relation to carcinogenesis and heart disease. The availability of selenium is limiting factor in glutathione peroxidase biosynthesis. The enzyme contains four identical subunits, each containing a selenium atom as selenocysteine. Four types of glutathione peroxidase have so far been identified (Flohe et al. 1976). The most extensively characterized form is the classical cellular enzyme GPx-1, which catalyzes the reduction of hydrogen peroxide and a wide range of organic peroxides, using glutathione as a reducing agent (Takahashi et al. 1987). Plasma glutathione peroxidase is derived principally from kidney and liver (Takahashi et al. 1987) and is immunologically distinct from GPx-1. The third form is a phospholipid hydroperoxide GPx that has the particular characteristic of acting directly on membrane-bound phospholipid hydroperoxides (Maiorino et al. 1991). Finally, a gastrointestinal glutathione peroxidase has recently been identified (Chu et al. 1993). This complex array of selenium-dependent enzymes acts synergistically with other antioxidants to protect cell membranes and constituents against the deleterious effects of reactive oxygen species (ROS) (Dhur et al. 1996).

Selenium Deficiency

Deficiency of selenium is reported to result in cardiomyopathy (Neve 1996), sudden death (Burk 1978), ECG abnormalities, hemorrhage of epicardial vessels, leading to the condition known as ‘mulberry heart disease’ in pigs (Harding 1960). Abnormalities in liver function, brain, heart, striated muscle, pancreas and genital tract have also been reported due to selenium deficiency (Rampal 1987). Selenium deficiency is known to cause “Keshan disease”. The precise mechanism by which selenium deficiency produces these consequences is unknown. Some authors have indicated that low selenium concentrations may facilitate the formation of lipid hydroperoxides, which could damage the vascular endothelium (Turk et al. 1980). Other studies have established that selenium affects prostaglandin biosynthesis, enhancing the thromboxane content of platelets and reducing prostacyclin.
production by the vascular endothelium (Neve 1996).

Selenium and cerebral ischemia

Selenium was reported to be helpful in slowing down the progression of neurodegeneration in Parkinsonism (Zafar et al. 2003). Selenium was reported to be helpful in protection against neurodegeneration in cerebral ischemia (Ansari et al. 2004). Paterson and Juurlink (1999) studied the impact of nutritional status of selenium on the prevention and outcome of stroke. These findings provided the mechanism that was able to explain both the inverse correlation between dietary protein and stroke mortality. Investigations to examine the role of nutritional intervention in neuroprotective strategies aimed at improving stroke outcome that accompany protein-energy malnutrition which have a definite role for antioxidant defenses in cerebral ischemia are required. Administration of sodium selenite either before or after global cerebral ischemia markedly reduced cerebral infarct size, marked decrease in mitochondrial lipid peroxidation, attenuated impairment in short-term memory and motor coordination. Rutin and garlic oil administrated before cerebral ischemia may scavenge reactive oxygen species and consequently attenuate global cerebral ischemia and reperfusion-induced cerebral injury whereas sodium selenite administrated before and after cerebral ischemia may be neuroprotective due to its antioxidant effect (Gupta et al. 2003).

Combinational studies with alpha-tocopherol acetate and selenium in patients with small ischemic insults evaluated which resulted in the decreased content of diene conjugates and malondialdehyde. While activity of superoxide dismutase, catalase, glutathione peroxidase and reductase was increased after administration of both these antioxidants during treatment of the brain circulation impairments (Dzhandzhgava and Shalarishvili 1991).

![Figure 16: Hypothetical model of Se transport via SePP and its uptake mechanism by the brain and other tissues.](image)

**Mechanistic Approach of Cellular & Molecular Mechanisms During Cerebral Ischemia**

Injury from ischemic stroke is the result of a complex series of cellular and molecular metabolic events that occur rapidly after the interruption of nutrient blood flow to a region of the brain. The duration, severity, and location of focal cerebral ischemia determine the extent of brain function and thus the severity of stroke. The molecular cascades that finally lay its hands on the molecule are interconnected and it is in this phase that either there is necrotic or apoptotic cell death depending on the route taken by the cell. A summary of the cascade of cellular and molecular events in ischemia progression are briefly discussed below.
Blood Brain Barrier (BBB)

Blood brain barrier (BBB) is a physiological barrier, functions as an etiologic component of many neurological diseases (Figure 17). An intact BBB may restrict the delivery of certain therapeutic substances to the brain but, at the same time, it restricts the entrance of a number of antioxidants during pathological conditions. Thus, measuring BBB function may be important to diagnose disease progression and monitor time-dependent changes in BBB integrity when chemotherapeutic penetration may be enhanced. Ischemia produces BBB permeability to large molecules and sustained cerebral edema only when the process damages blood vessels, astrocytes, and neuronal necrosis alone is insufficient. Ischemia causes disruption of the blood-brain barrier (BBB) that leads to the formation of vasogenic brain edema. BBB opening is enhanced by pinocytotic vesicle formation, which may be induced after transient focal ischemia by several mechanisms including nitric oxide production, release of neurotransmitters, inflammatory mediators and hemodynamic alterations (Cipolla et al. 2004). Finally, as noted already, when neurons are lost, it is permanent since these cells are differentiated to the point where they can no longer undergo cell division.

Brain edema is a life-threatening complication of cerebral infarction. The molecular cascade initiated by cerebral ischemia includes the loss of membrane ionic pumps and cell swelling. Secondary formation of free radicals and proteases disrupts brain-cell membranes (BBB), causing irreversible damage. Ischemic brain edema is a combination of two major types of edema: cytotoxic (cellular) and vasogenic (Fishman 1992). Cytotoxic edema evolves over minutes to hours and may be reversible, while the vasogenic phase occurs over hours to days, and is considered an irreversibly damaging process. Cytotoxic edema is characterized by swelling of all the cellular elements of the brain or fluid accumulated within the cell as a result of cell injury (Figure 18). In the presence of acute cerebral ischemia, neurons, glia (indicated by astrocytes), and endothelial cells swell within minutes of hypoxia due to failure of ATP-dependent ion (sodium and calcium) transport. With the rapid accumulation of sodium within cells, water follows to maintain osmotic equilibrium. Increased intracellular sodium, chlorine, glutamate, lactate and finally it is calcium, which activates phospholipases and the release of arachidonic acid, leading to the release of oxygen-derived free radicals and infarction. Therefore, recovery of Na⁺ K⁺ ATPase activity coincides with restoration of cerebral edema after brain hypoxia–ischemia. Neurotoxic edema is a subtype of cytotoxic edema caused by high levels of excitatory amino acids i.e. there is increased release of glutamate.

Vasogenic edema is characterized by an increase in extracellular fluid volume due to increased permeability of brain capillary endothelial cells to macromolecular serum proteins (e.g., albumin) or as fluid originating from blood vessels and accumulating around cells. Normally, tight endothelial cell junctions limit the entry of plasma protein-containing fluid into the extracellular space. But in the presence of...
massive injury there is increased permeability of brain capillary endothelial cells to large molecules i.e. increased solutes and water content enters the brain from disrupted BBB. Vasogenic edema can displace the brain hemisphere and, when severe, lead to cerebral herniation.

**The Ischemic Penumbra**

Within the ischemic cerebrovascular bed, there are two major zones of injury: the core ischemic zone and the "ischemic penumbra" (the term generally used to define ischemic but still viable cerebral tissue) (Figure 19). In the core zone, which is an area of severe ischemia (blood flow below 10% to 25%), the loss of inadequate supply of oxygen and glucose results in rapid depletion of energy stores. Brain cells within the penumbra, is the area in which infarction is evolving, may remain viable for several hours. That is because the penumbral zone is supplied with blood by collateral arteries anastomosing with branches of the occluded vascular tree.

However, even cells in this region will die if reperfusion is not established during the early hours since collateral circulation is inadequate to maintain the neuronal demand for oxygen and glucose indefinitely. The penumbra is where pharmacologic interventions are most likely to be effective. However, it may also be possible to salvage cells within the severely ischemic core zone. Although severe ischemia kills selectively vulnerable neurons, glial cells may be spared if blood flow is restored early. Therefore, timely recanalization of the occluded vessel should theoretically restore perfusion in both the penumbra and in the severely ischemic core. Partial recanalization should markedly reduce the size of the penumbra as well.

**Inadequate Energy Supply**

Inadequate energy supply is the first primary step of ischemia. Lack of glucose and oxygen deplete the cellular energy stores required to maintain electrical potentials and ion gradients. In ischemic brain tissue, the membrane that surrounds each affected neuron becomes "leaky," and the cell loses potassium and adenosine triphosphate (ATP), the tissue's medium for energy exchange. 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. 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. With respect to the latter, anaerobic glycolytic pathways 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 onset of ischemia. Toxicity of hydrogen ions, especially their ability to
facilitate ferrous-iron-mediated free-radical mechanisms, appears to irreversibly affect neuronal integrity.

Once ATP levels fall which is an important indicator of possible changes in mitochondrial function during cerebral ischemia and reperfusion (Sims and Anderson 2002), energy dependent processes that include the maintenance of ion gradients, the contraction of muscle, secretion of transmitters, and the maintenance of normal regulation of calcium required for the coordination of calcium signals will inevitably become disordered. Once ionic gradients dissipate, intracellular osmolarity cannot be maintained, and cells will swell and die by a process of necrosis. During the impaired glycogen delivery (10-26% of non-ischemic values), major ATP (60-90%) and phosphocreatine (16-28%) are severely altered during focal ischemia than global ischemia (Hata et al. 2000).

OXIDATIVE STRESS

The pretentious organ brain, comprising 2% of the body weight, utilizes 20% of the oxygen consumed by the body is highly endangered with regard to the generation and detoxification of reactive oxygen species (ROS) that are produced continuously during oxidative metabolism. Defense against the toxic effects of reactive oxygen species is an essential task within the brain. The drawback of the neuronal population is that the brain cells do not regenerate and the loss of neurons cannot be compensated by the generation of new neurons. Nevertheless, the brain is able to function during a long human life, indicating the presence of an effective antioxidant system in brain. Experimental ischemia and reperfusion models have been thoroughly studied and the cumulative evidence suggests involvement of oxygen radicals in the pathogenesis of ischemic lesions. The balance between the generation of ROS and antioxidative processes is one of the basic perpetrators of cerebral ischemic cascade, which further gives rise to a complex array of disturbed neuronal equilibrium. The antioxidant status during cerebral ischemia is extensively studied. CNS takes more share of oxidative stress than other organs (Floyd and Carney 1992) as it has high concentrations of polyunsaturated fatty acids (PUFA), which are much prone to ROS attack. Adequate oxygenation is critical to cellular viability. The paradox of reperfusion injury can be understood in terms of counter adaptive changes occurring in cerebral ischemia that predispose to cellular dysfunction, apoptosis and necrosis during reperfusion. Antioxidant changes reflect an altered redox balance in several pathological states (Gutteridge and Halliwell 2000). The measurement of antioxidant activity has been performed to estimate the extent of oxidative stress. Increased ROS generation also occurs in the penumbra surrounding the infarct (Solenski et al. 1997), which can contribute to progressive damage in penumbral tissue (Chan 1996, Love 1999).

Figure 20: The recycling of glutathione. Hydrogen peroxide (H₂O₂) is reduced to water (H₂O), while glutathione (GSH) is oxidized to glutathione disulfide (GSSG). Each cell has a limited amount of GSH, so GSSG must be recycled to the reduced state (GSH) to maintain protection against H₂O₂. Glutathione reductase (GR) uses electrons from the oxidation of NADH to convert GSSG into GSH.
Occluded vessels in brain regions that are supplied with oxygen reduce cerebral blood flow. When reperfusion occurs, white blood cells re-enter a previously hypoperfused region via returning blood, they can occlude small vessels, producing additional ischemia. Leucocytes release toxic products that lead to free radical and cytokine formation. Reoxygenation during reperfusion provides a substrate for numerous enzymatic oxidation reactions. Mitochondria produce superoxide anion radicals and hydrogen peroxide ($H_2O_2$) under normal physiological conditions. These constantly produced reactive oxygen species (ROS) are scavenged by superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase. SOD specifically processes superoxide anion ($O_2^-$) and produces $H_2O_2$, which is then detoxified by catalase or GSHPx, and finally changed to water and superoxide. Hydroxyl radicals (•OH) may be generated from $H_2O_2$ through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow HO + Fe^{3+} +$ •OH). Other small molecular antioxidants, including glutathione (GSH), ascorbic acid (Vit C), and α-tocopherol, are also involved in the detoxification of free radicals (Figure 20). Reperfusion after ischemia causes overproduction of ROS in mitochondria, and consumption of endogenous antioxidants by these radicals may lead to a dramatic rise in intracellular ROS. It has been demonstrated in numerous studies that ROS are directly involved with cellular macromolecules such as lipids, proteins, and nucleic acids in oxidative damage in ischemic tissues, which leads to cell death. Recent studies have provided evidence that indirect signaling pathways mediated by ROS can also cause cellular damage and death in cerebral ischemia and reperfusion (Ursu and Clarkson 2003, Sugawara et al. 2004).

Protein carbonyls, markers of protein oxidation, are also found to be increased both in the ischemic core and in the surrounding penumbra area during ischemia with a further augment during reperfusion only in the latter. Nitric oxide (NO), a free radical, has a double role during ischemia in relationship to the isoenzyme of NO synthase that is activated. NO produced by the constitutive endothelium nitric oxide synthase has a vasodilatative effect and hence it is neuroprotective. While NO from neuronal nitric oxide and inducible nitric oxide synthases contained in microglia and endothelium has been shown to be neurotoxic, in part by reacting with superoxide leading to the highly reactive peroxynitrite (Chan 2001).

Both pre and postsynaptic sites may be involved during ischemia by ROS (Chan 2004), since $H_2O_2$ can inhibit neurotransmitter release (Chen et al. 2001) and can cause hyperpolarization of CA1 pyramidal neurons (Seutin et al. 1995). It is also involved in the modulation of synaptic plasticity (Auerbach and Segal 1997, Klann et al. 1998), possibly mediated by alterations in intracellular signaling pathways, including activation of certain kinases (Klann and Thiel 1999). It is generated during normal mitochondrial respiration from dismutation of the superoxide radical (Boveris and Chance 1973, Halliwell 1992). Pathological consequences of $H_2O_2$ elevation are normally prevented by the endogenous antioxidant network, which includes the $H_2O_2$ scavenging enzymes glutathione (GSH) peroxidase and catalase (Cohen 1994, Desaghet et al. 1996, Sokolova et al. 2001). Modulatory, as well as pathological consequences of $H_2O_2$ are often mediated by the hydroxyl radical (•OH) rather than by $H_2O_2$ per se (Avshalumov et al. 2000, Halliwell 1992, Pellmar 1986). Whereas the enzymes superoxide dismutase (SOD), GSH peroxidase, and catalase regulate cellular levels of •$O_2^-$ and $H_2O_2$, there are no analogous enzymes for the highly reactive •OH radical. Instead, •OH radical management depends on the endogenous antioxidants ascorbate and GSH (Cohen 1994, Rice 2000). Extracellular glutamate levels increase 6-30 folds during ischemia, resulting in concentrations that can exceed
Review of literature

1 mM (Benveniste et al. 1984, 1991). ROS production during reperfusion is proportional to the increase in extracellular glutamate during the ischemic period (Morimoto et al. 1996). H2O2-induced neuronal apoptosis was due to increased extracellular glutamate concentration, N-methyl-D-aspartate (NMDA) receptor activation, and increased ROS production (Mailly et al. 1999).

**Phospholipase A2**

Phospholipid degradation is an important promoter of neuronal death after transient cerebral ischemia. Phospholipid hydrolysis by phospholipase A2 (PLA2) after transient cerebral ischemia releases arachidonic acid (AA) whose metabolism results in formation of reactive oxygen species, lipid peroxides, and toxic aldehydes malondialdehyde, 4-hydroxynonenal, and acrolein. Increased PLA2 immunoreactivity demonstrated a precise overlap with neuropathological changes in several types of CNS injury, including focal and global cerebral ischemia. PLA2 immunoreactivity was increased in the hippocampal CA1 region undergoing neuronal death. PLA2 isozymes occur in multiple forms in the mammalian cell and are classified as calcium independent, cytosolic (cPLA2), and secretory (sPLA2) (Murakami et al. 2002). cPLA2 is stimulated by micromolar Ca2+ concentrations, whereas sPLA2 requires millimolar Ca2+ for optimal activity. In the absence of Ca2+, there was very little PLA2 activity, indicating that there is no significant amount of the Ca2+-independent form. There was very little increase in PLA2 activity at 10-100 μM Ca2+, the range where cPLA2 is activated, suggesting that this isoform is also present in gerbil hippocampus at very low concentrations (Yang et al. 1999).

Treatment of rat brain hippocampal slices with antimalarial drugs (non specific cPLA2 inhibitors), arachidonyl trifluoromethyl ketone (a specific cPLA2 inhibitor), or surfactin (a non specific cPLA2 inhibitor) not only inhibits cPLA2 activity but blocks neurodegeneration, suggesting cPLA2 inhibitors can be used as neuroprotectants and anti-inflammatory agents in neurodegeneration (Farooqui et al. 2004). PLA2 inhibitors prevent hypoxic nuclear shrinkage in cells and cell death. The elevation of PLA2 activity and translocation of intracellular PLA2 to the nucleus. Knock-down of the Ca2+ independent PLA2 delayed nuclear shrinkage and cell death which indicate that Ca2+ independent PLA2 is crucial for a caspase-independent cell death signaling pathway leading to nuclear shrinkage (Shinzawa and Tsujimoto 2003).

**STRESS PROTEINS**

Various stress proteins are expressed in response to cerebral ischemia, among which heat shock proteins (Hsp's) and metallothioneins (MT) are of prime importance.

**CEREBRAL ISCHEMIA AND HSP'S**

In response to ischemia, organisms synthesize a certain set of proteins, which prevents perturbation the protein structure they are called as heat shock proteins (Hsp's). They belong to an extensive family of proteins, which are highly conserved in eukaryotes (Hunt and Morimoto 1985). They act as chaperones involved in the regular formation, folding and assembly of protein chains and in the translocation of newly formed proteins through endoplasmic reticulum membranes. These molecular chaperones are also defined as “proteins that bind to and stabilize an otherwise unstable conformer of another protein and by controlled binding and release of the substrate protein, facilitates its correct fate in vivo: be it folding, oligomeric assembly, transport to a particular subcellular compartment, or controlled switching between active/inactive conformations” (Hendrick and Hart 1996). Hsp's are
induced during stressful conditions in a variety of systems including brain and exert neuroprotective function (Massa et al. 1996). In mammalian brain, 3 main Hsp's are expressed or induced after stress; the Hsp90 (Quraishi and Brown 1995), Hsp70's and the low molecular weight Hsp's (Ewing et al. 1992).

During cerebral ischemia, Hsp70, which is not normally present, is induced in the mammalian brain (Abe and Kogure 1993). Hsp70, a marker of neuronal injury, is highly expressed in different neural cell regions affected by brain insults (Lee et al. 2001). During middle cerebral artery occlusion in the rats, inductions of Hsp70 mRNA and protein within the ipsilateral brain area was reported with the severity depending upon the duration of MCAO time (Memezawa et al. 1992, Lee et al. 2001). During global cerebral ischemia, strong induction of Hsp70 was detected mainly in the dentate gyrus, CA3 and CA1 of the hippocampus neurons after 24 hr post ischemia (Planas et al. 1995). Hsp70 increases the resistance of non-neural cells in culture to apoptosis (Samali and Cotter 1996), indicating that Hsp70 participates in mechanisms that inhibit apoptosis (Dix et al. 1996). It is worth exploring whether stimulation of Hsp70 expression can prevent or reduce apoptotic cell loss induced in the brain following insults. Hsp70 induction increases the number of surviving neurons in rat hippocampal neuron primary culture as well as the tolerance of hippocampal neurons to ischemic injury (Kirino et al. 1991). Thereby preconditioning may limit the development of arterial hypotension, cerebral monoamine overload, augmented the production of interleukins, cytokines in the plasma and neuronal damage (Yang and Lin 1999). Gene therapy with Hsp70 improves neuron survival after focal cerebral ischemia. Cytoprotective effects of Hsp70 in a number of in vitro studies are reported. Both heat shock and Hsp70 overexpression protect neurons against glutamate-mediated toxicity (Lowenstein et al. 1991) sublethal heat shock (Narasimhan et al. 1996), glucose or oxygen deprivation (Xu and Giffard 1997) and simulated ischemia (Amin et al. 1996).

METALLOTHIONEINS AND CEREBRAL ISCHEMIA

Significant contribution in understanding the proficiency of living organisms to cope with metal ions and use them in cellular metabolism and growth originated with the realization that metal accumulation and function are invariably linked to the existence of specific metal binding proteins, metallothioneins. Metallothioneins are a class of cysteine-containing, low molecular weight, ubiquitous intracellular proteins with high affinity for metals nearly discovered 47 years ago by Margoshes and Vallee. Metallothioneins (MTs) are present in 4 isoforms as MT-1, MT-2, MT-3, and MT-4 (Harmer 1986). MT-1 and MT-2 are found in all organs, whereas MT-3 is expressed mainly in brain (Palmiter et al. 1992), and MT-4 is most abundant in certain stratified squamous epithelial tissues (Quaife et al. 1994).

MTs are reservoirs for zinc and copper (in case of deficiency), serve as important regulators of metal homeostasis and as a source of zinc incorporated into proteins, including transcription factors (Palmiter 1998), and are able to prevent zinc deficiency and toxicity in vivo (Dalton et al. 1996). It is proposed to function as detoxifying agents of other reactive metals and free radicals (Koh et al. 1996). Both zinc toxicity (Koh et al. 1996) and oxidative stress (Sato and Bremner 1993) contribute to ischemia-induced cell death. Zinc is an important cofactor in many proteins; high levels of free zinc are deleterious to many cells including those in the central nervous system. Cysteine residues of MT may serve as an expandable target for reactive oxygen species (Lazo et al. 1995). "House keeping" functions of MTs are implicated in metal metabolism, cellular
Review of literature

repair processes, growth and differentiation, where they likely serve as a source of zinc for newly synthesized apoenzymes and for regulator molecules of gene expression. MT might function as a thiol donor and free radical scavenger, thus providing protection against free radical damage (Lazo et al. 1995). Rapid induction of MT-1 mRNA and protein in microvascular endothelial cells and glial cells before the peak formation of cerebral edema, implicates a role for MT-1 in blood-brain barrier integrity after cerebral ischemia (Campagne et al. 2000).

Metallothioneins are important neuroprotective proteins in focal cerebral ischemia (Trendelenburg et al. 2002). MT-III serves as a neuromodulator (Palmiter 1992). Zinc is released at glutamnergic synapses in response to depolarization and possess characteristics of a neuromodulator for ionotropic glutamate receptors (Palmiter 1995). Selective neuronal cell death after global cerebral ischemia is associated with the accumulation of zinc and that chelation of zinc with calcium-EDTA decreases this degeneration (Koh et al. 1996). MT-1 and MT-2 increase after an ischemic insult. MT-1 transgenic (MT-TG) mice have a reduced infarct size and reduced sensorimotor defects after cerebral ischemia (Campagne et al. 1999). When the ischemic insult induces cellular accumulation of free zinc, the MT-1 molecules are most likely fully saturated with zinc. It may be that the accumulation of zinc released from synaptic vesicles and zinc released from MTs after oxidative stress could activate transcription of MT-1 through metal transcription factor-1. MT-3 protects CA3 hippocampal neurons against cell death induced by seizures, a situation where massive release of zinc from mossy fiber terminals occurs. In addition, cells expressing MTs are resistant to toxic effects of NO. After cerebral ischemia, the activity of inducible NO synthase and the production of NO are increased and significantly contribute to infarct development (Pereira et al. 1990), a close correlation between increased expression levels of MT-1 and protection against cerebral ischemia and reperfusion is known. Based on the potential role of MT-1 as a modulator of zinc toxicity and cellular redox state, indicate MTs as novel target molecules for treatment of stroke and further support a role of free radicals and or heavy metal toxicity in the pathogenesis of stroke. Whether the immune-regulatory properties, the zinc-binding properties, the free radical-scavenging properties, or the redox properties of MTs play an important role in protection against ischemia-reperfusion is still unclear.

CEREBRAL ISCHEMIA & CYTOCHROME P450 (CytP450)

Cytochrome P450 enzymes (CytP450s), are membrane bound, heme-containing family of proteins catalyze the formation of biologically important lipid signaling molecules, including eicosanoids and steroid hormones, and play important roles in cellular adaptation to oxidative stress and environmental toxins. Among the major milestones in the P450 field was their expression in brain parenchymal cells and cerebral blood vessels, their products being endogenous constituents of brain imparting their roles in various brain functions and neurological diseases. They are classified depending on their cellular localization into microsomal P450s, which are present in endoplasmic reticulum and mitochondria (therefore associated with inner mitochondrial membrane). Microsomal P450s use molecular oxygen to oxidize substrates by transferring one atom of oxygen to their substrate and the other to water. This action requires electron transfer from nicotinamide adenine dinucleotide phosphate (NADPH) via two cofactors, the flavoprotein cofactor P450-reductase and cytochrome b5. Highest concentrations of P450s are in liver, adrenals, gonads and placenta. CytP450 in brain is between 0.5% and 10% of their level in liver, whereas in specific neuronal populations higher levels than hepatocytes is reported. Hepatic P450...
metabolize and impact overall drug levels thereby affect distant targets in endocrine fashion which is opposite for brain, the generated products remain contained within the brain and are specific for brain functions. Brain \( P_{450} \)'s offers neuroprotection, control of cerebral blood flow, formation of neuroactive steroids, temperature control, neuropeptide release, maintenance of brain cholesterol homeostasis, elimination of retinoids from CNS, regulation of neurotransmitter levels and other functions important in brain physiology, development and disease. Cytochrome \( P_{450} \) genes are classified into families and subfamilies based on sequence homology. \( P_{450} \) isoforms involved in cholesterol metabolism and steroidogenesis have also been detected in brain, including \( P_{450} \) 7B, \( P_{450} \) 11A1, \( P_{450} \) 11B, \( P_{450} \) 26A and B, \( P_{450} \) 17, \( P_{450} \) 21 and \( P_{450} \) 46.

Unlike peripheral tissue, where excess cholesterol is cleared via lipoprotein particles, the blood brain barrier prevents cholesterol removal from brain via this mechanism. In brain, cholesterol is removed after hydroxylation to \( 24R \) and \( 24S \)-hydroxycholesterol via cholesterol 24-hydroxylase (\( P_{450} \) 46A1), which is 100 fold at a higher level than in liver. Elimination of cholesterol products is an important protective mechanism since these products possess neurotoxic effects and have been implicated in the pathogenesis of cerebral ischemic stroke, Alzheimer's disease. Brain and liver \( P_{450} \)s respond differently to the same inducers, likely reflecting differences in molecular regulation. Many drug-metabolizing \( P_{450} \)s are expressed at the blood-brain interface and in brain regions not protected by the blood brain barrier, such as the choroid plexus, the median eminence, area postrema and the posterior pituitary. This lead to the notion that the presence of \( P_{450} \) in these locations represents an 'enzymatic barrier' evolved to protect the brain from toxic chemicals. The brain and cerebral blood vessels express arachidonic acid (AA) metabolizing \( P_{450} \) enzymes, and both epoxycisatrienoic (EETs) and hydroxyecosatetraenoic acids (HETEs) are produced in brain parenchymal tissue and blood vessels. The major \( P_{450} \) AA metabolite produced in blood vessels is 20-hydroxycisastatetraenoic acid (20-HETE), a potent vasoconstrictor in the cerebral circulation and a product of the \( P_{450} \) 4A family. 20-HETE plays an important role in the mechanism underlying autoregulation of cerebral blood flow (CBF) and contributes to the vasodilator actions of nitric oxide in the cerebral circulation. EETs on the other hand are potent dilators of cerebral blood vessels, and are produced in brain by astrocytes via the action of \( P_{450} \) epoxygenases. Certain \( P_{450} \) isoforms involved in AA metabolism can be induced in brain, and that AA metabolites produced by these isoforms may play a neuroprotective role against ischemic cell death as the studies on \( P_{450} \) gene expression of a neuroprotective strategy such as ischemic preconditioning (IPC), whereby mild sublethal ischemic stress confers protection against subsequent severe ischemic insult, ischemic preconditioning upregulates a battery of defense mechanisms and switches cells to a more protected phenotype, leading to enhanced tolerance against eminent lethal ischemia.

**Mitochondrial Changes**

Mitochondrion lies at the heart of cell life and cell death. Mitochondria are essential to maintain the battle against entropy that is necessary to sustain life. They provide the energy required for almost all cellular processes all the processes central to sophisticated life. Therefore, mitochondrial dysfunction will lead to disease, ranging from the subtle alterations in function in tissues that may manifest as disease and illness, to major defects in tissue function that may lead to major handicap or death. Mitochondria have a crucial role in both apoptotic and necrotic cell death. Antioxidants inhibit DNA fragmentation.
Review of literature

Figure 21: Structure & function of the mitochondrion. (A) (i) and (ii) show 3-D reconstructions of mitochondrial structures from EM tomography. The mitochondrion in (i) came from the dendritic tree of a cerebellar Purkinje cell. The spots in (ii) show contact sites between inner and outer membranes of rod photoreceptor mitochondria. In (B) is shown a cartoon to represent the functional components of the mitochondrion and (C) shows a more detailed scheme of the respiratory chain.

and PARP cleavage, suggesting that ROS are responsible for these manifestations. Reperfusion itself enhances expression of CD95L (APO-I/Fas), a transmembrane protein that induces apoptosis by ligand binding and subsequent activation of caspases (Vogt et al. 1998).

The chemiosmotic principle

The key enzymatic components of the mitochondria are the citric acid or tricarboxylic acid (TCA) cycle and the respiratory or electron transport chain. The enzyme system of the TCA cycle breaks down carbon substrates acetyl CoA, derived from pyruvate, fatty acid and amino acid breakdown to generate CO₂ and in the process to reduce NAD⁺ to NADH and FAD²⁺ to FADH₂.

These intermediates provide reducing equivalents to the respiratory chain which consists of a series of enzyme systems coupled together and described as Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), Complex III (ubiquinol cytochrome c reductase) and Complex IV (cytochrome c oxidase). These are all complex membrane spanning enzyme systems consisting of many protein subunits. Operationally, there is a transfer of energy between the intermediates of the chain moving progressively energetically 'downhill' from a reduced to an oxidized state. Electrons are transferred from NADH and FADH₂ to Complexes I, and Complex II respectively, and these each transfer electrons to ubiquinone which shuttles electrons to Complex III. Cytochrome c then shuttles electrons to complex IV. All of these play a major role in catalyzing the redox reactions.

Alterations in mitochondrial respiration develop in focal ischemia induced after 2h of middle cerebral artery occlusion (MCAO). Stimulated respiratory activity was reduced by 45-60% in focal tissue and by 15-40% in perifocal regions (Anderson and Sims 1999). Respiration by both NADH-linked and FADH₂-linked substrates is impaired in focal (core) and perifocal tissue. Larger alterations in FADH₂-linked respiration supported by succinate (Anderson and Sims 1999,2000), suggest an additional alteration affecting components involved in succinate oxidation (Figure 21).

Inducers of Neuronal damage: the role of free radical species

Mitochondria, the main sustainer of cellular energy and metabolism, play a central role in cell death by controlling cellular energy metabolism (Chan 2004). Its dysfunction in
ischemic conditions can be attributed mainly to either inhibition of adenine nucleotide translocase, reduced activity of complex I and III alteration of the membrane fluidity or calcium overload. The most prominent pro-apoptotic factor released from mitochondria is cytochrome c (cyt c) (Andrabi et al. 2004). Release of pro-apoptotic factors may occur with or without opening of the mitochondrial permeability transition (MPT) pore. Oxidative stress, often reflecting mitochondrial generation of free radicals that plays a major role in cerebral ischemic injury.

Mitochondria represent not only a major source of ROS generation, but also a major target of ROS induced damage (Chan 2004). The molecular targets damaged by mitochondrially derived ROS, lipid peroxidation, ion channel modification, and DNA damage have all been demonstrated in models employing exogenous oxidizing agents (Wakatsuki et al. 1999). Mitochondrial ROS may also inhibit one or more of the components of the respiratory chain, further accelerating the rate of superoxide formation (Starkov et al. 2004). Mitochondrially produced superoxide may directly lead to reduced complex I activity. Ischemia and reperfusion is associated with decreased electron transfer activity and oxidation of mitochondrial membranes. Based on the above mentioned studies workers proposed a model in which there is feedback between ROS production by mitochondria and calcium release from nearby endoplasmic reticulum leading to increasing mitochondrial calcium load and culminating in irreversible loss of mitochondrial membrane potential and cell death.

Mitochondrial membrane potential

The mitochondrial membrane potential lies at the heart of all the major bioenergetic functions of the mitochondrion. From the manufacture of ATP to accumulation of calcium, it provides a force that drives the influx of protons or of calcium that simply move into the mitochondria down their electrochemical potential gradients. It is important to understand that mitochondrial membrane potential is normally maintained by cellular respiration. A loss of mitochondrial potential may be a reaction of several different mechanisms—an inhibition of respiration, a failure of provision of substrate or some kind of uncoupling mechanism that shunts the proton circuit and so dissipates the potential. The rate at which mitochondrial potential is dissipated in response to these different processes may also vary considerably between cells.

Cytochrome c (cyt c): role in electron transport, release and regulation of apoptosis

Cyt c is a 13 kDa nuclear encoded protein that is highly conserved among species. It is imported into mitochondria as an unfolded apoprotein without requiring ATP or a high mitochondrial membrane potential. Covalent attachment of a reduced heme group results in globular holo-cyt c, which moves freely in the intermembrane space to transport electrons from complex III to complex IV of the respiratory chain during oxidative phosphorylation. In addition to its role as an electron carrier, holo-cyt c is a
mitochondrial factor central to the induction of the apoptotic cascade by relocating from the intermembrane space of mitochondria to the cytosol where it participates in formation of the apoptosome that activates caspase 9 and subsequently caspase 3 (Andrabi et al. 2004). Mitochondrial contribution to cerebral ischemic damage is not fully elucidated because many mitochondrial properties can only be assessed indirectly in the intact brain. Studies of cells in culture and of isolated brain mitochondria exposed to ischemia-like conditions have been used to more directly evaluate mitochondrial response to stress (Sims and Anderson 2000).

Translocation of cyt c from mitochondria to cytosol is a marker of a pathological increase in the permeability of one or both mitochondrial membranes that leads to mitochondrial dysfunction and apoptotic or necrotic cell death that has been observed after transient forebrain ischemia. It is now appreciated that cyt c release occurs by at least two mechanisms: from MPT pore opening or without MPT (Figure 22).

However, in some cases of apoptosis, cyt c release occurs before a loss of mitochondrial membrane potential and proceeds in the presence of MPT inhibitors. It has also been reported that mitochondrial membrane hyperpolarization may precede cyt c release.

After ischemia reperfusion mitochondrial respiratory dysfunction occurs which can trigger apoptosis via mitochondrial permeability that causes the liberation of apoptogenic factors from mitochondria. The release of cytochrome c and other mitochondrial proteins from the intermembrane space to the cytosol is commonly a critical step in the death of cells by apoptosis that can result from disruption of the outer mitochondrial membrane due to swelling associated with induction of the permeability transition pore. However, alternative mechanisms that are independent of the permeability transition pore and of mitochondrial swelling are apparently often involved (Sasaki et al. 2000, Schaller and Graf 2004). Bax and some other member of the bcl-family of proteins have been implicated in this release process. After 4 hours of reperfusion (90 min of MCAO), cytochrome c was seen in the cytoplasm of some neurons (Babu et al. 2000). By 24 hours of reperfusion, many cells throughout the MCA territory were affected. In some of the affected cells, the staining was associated with DNA fragmentation suggesting apoptosis in permanent and transient focal ischemia in rats and mice (Fujimura et al. 2000, Schaller and Graf 2004).

Caspase Activation & DNA damage

Figure 23: Upstream initiator caspases are activated during the initiation of the cell-death cascade. They contain activation or binding prodomain (white), a large subunit (orange), and a small subunit (yellow). Activated upstream caspases have autocatalytic activity and activate downstream effector caspases, which have a short prodomain (blue), as well as a large subunit (purple) and a short subunit (green). Downstream caspases mediate many of the classic phenomena of apoptotic cell death.

Cytochrome c loss from the mitochondrial matrix leads to activation of caspases, major executioners in the apoptotic program. Caspases are cysteine proteases (cysteine-
dependent, aspartate-specific proteases) acting in a proteolytic cascade, targeting key homeostatic and structural proteins leading to DNA fragmentation and cell death (Kroemer et al. 1998). Cytochrome c interacts with APAF-1 (apoptosis protease-activating factor 1) and caspase-9, leading further to activation of downstream caspases (Green and Reed 1998). The activity of caspase-3-like activity increases progressively between 1 and 24 h of reperfusion period and inactive (32 kDa proform) is cleaved to active forms (e.g. 17 kDa) along with increased immunoreactivity of 'active' caspase-3 in tissue sections (Hu et al. 2000). Caspase-3, -8, and -9 are activated by ischemia, possibly through Ca²⁺-induced activation of other proteases. Serine protease inhibitors suppress cytochrome c-mediated caspase-9 activation and apoptosis during reperfusion (Schaller and Graf 2003).

Till now 14 members of the caspase family have been identified, 11 of which are present in humans (Yuan et al. 1993). Caspases directly and indirectly orchestrate the morphologic changes of the cell during apoptosis. Caspases exist as latent precursors, which, when activated, initiate the death program by destroying key components of the cellular infrastructure and activating factors that mediate damage to the cells. Procaspases are composed of p10 and p20 subunits and an N-terminal recruitment domain. Active caspases are heterotetramers consisting of two p10 and two p20 subunits derived from two procaspase molecules. Caspase-3 activation developed much later in a model of permanent middle cerebral artery occlusion but activation of another caspase, caspase-8, was seen within the first 8 h of occlusion under ischemic conditions (Velier et al. 1999) (Figure 23).

Caspase-3 is implicated in cerebral ischemia as evidenced by increased rat caspase-3 mRNA 1 h after the induction of permanent ischemia (Gill et al. 2002). Caspases are effective in a large number of substrates, acting as scissors on structural and vital proteins, including PARP. PARP-1 over activation depletes NAD⁺ and ATP and it is associated with necrosis. Whereas PARP-1 cleavage results in the products of 89 and 28 KDa is characteristic of caspase activation and programmed/apoptotic cell death (Ha et al. 2000, Warner et al. 2004).

Recent researches have shown that DNA is a target of oxidative attack during reperfusion following cerebral ischemia. It is currently believed that peroxynitrite, superoxide and particularly hydroxyl radical can produce different types of oxidative DNA damage (Chen et al. 2001). DNA ligation is dependent on the presence of poly (ADP-ribose) polymerase (PARP) because an inhibition of PARP alters the rate of repair (LaPlaca et al. 1999). Breaks in DNA strands after ischemic damage activate PARP and cause the depletion of nicotinamide adenine dinucleotide (NAD, a substrate of PARP) and ATP. Base-excision repair is disrupted with PARP-bound DNA. However, the increase of sister chromatid exchange in PARP-deficient cells suggests that binding of PARP to DNA is required for orderly ligation of DNA. Activation of PARP enhances necrosis but reduces apoptosis after ischemic damage (Nouspikel and Hanawalt 2000). Thus, the participation of PARP in gene repair in the brain may be a double-edged sword. Gene-repair pathways are probably present and functional in the brain, because several proteins that are involved in all types of repair are induced by brain injury and cerebral ischemia (Liu et al. 1996).

Evidence of DNA repair has been presented after cerebral ischemia (Schulz et al. 1995). Ischemia-induced oxidative DNA lesions in the transcribed strand of the c-fos gene are repaired initially more slowly than the non-transcribed strand, but almost all are repaired within 2 to 3 hours (Liu et al. 1996). The rate of oxidative DNA lesion repair in nuclear and mitochondrial DNA might be similar (Liu et al. 1996). Activation of caspases and the production of
oligonucleosomal DNA can be detected as “DNA ladders” on gel electrophoresis with more extended periods of ischemia/reperfusion (Guegan and Sola 2000).

In a nutshell, the experimental proceedings discussed in the following pages employ the therapeutic potentials of various drugs for the prophylactic aspect of focal cerebral ischemic damage. The aim of the study will be to evaluate the neuroprotective efficacy of various drugs against the ischemic damage at various points of ischemic cascade, which will be the prime cult.
Figure 24: Demonstrating the molecular mechanisms involved during cerebral ischemia.