EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 12
277-285
Year: 2017

PATHOPHYSIOLOGICAL REVIEW ON STROKE AND ITS POSSIBLE TREATMENTS

Harsimran Singh*, Devinder Kaur, Satvir Singh and Nitin Bansal

Department of Pharmacology, ASBASJSM College of Pharmacy, Bela.

***Corresponding Author: Harsimran Singh**
Department of Pharmacology, ASBASJSM College of Pharmacy, Bela.

Article Received on 01/10/2017 **Article Revised on 22/10/2017** **Article Accepted on 14/11/2017**

ABSTRACT
Stroke is considered as one of the most life threatening neurological disorder. It is among the leading death causing disease in the world. It leads to cerebral ischemia which further can cause irreversible damage to the brain. This damage can affect various functions of the brain like cognition, learning, loss of senses, loss of motor activities or abnormal motor activities, etc. Stroke leads to the excitotoxicity, acidotoxicity and ionic imbalance, peri-infarct depolarization, oxidative and nitrative stress, inflammation and apoptosis, etc. The treatments for Stroke are very less, if available they are not well known. In this review, we will give a complete incite about the pathophysiology and possible treatments for stroke.

KEYWORDS: Stroke, Ischemia, Pathophysiology, Treatments.

INTRODUCTION

Stroke is considered the most common life-threatening neurological disorder and is the third leading cause of death in the United States**.** Although stroke is more disabling than lethal, it is estimated that there were over 150,000 deaths attributable to stroke in 1979 (American Heart Association, 1991). About one-half of the individuals who survive a stroke have significant persisting neurological impairment and physical disability. Currently, over 2 million stroke survivors and 400,000 stroke patients are discharged annually in the United States (American Heart Association, 1991). Thus, the economic costs of stroke total billions of dollars.[1]

Ischemia is defined as diminution of cerebral blood flow (CBF) to a critical threshold that propagates brain damage involving the entire brain or a selective region. Global cerebral ischemia entails diminution in CBF over the entire brain, encountered clinically as sequelae during extracorporeal circulation following CA from ventricular fibrillation or asystole that lasts 5 to 10 minutes. Global ischemia from CA results in a predictable pattern of histologic injury in which specific neuronal populations are affected (selective ischemic necrosis).[2] Although reperfusion restores CBF, it can lead to secondary brain injury from influx of neutrophils and to increases in reactive oxygen species (ROS), cerebral edema, and hemorrhage. Elevated levels of ROS may lead to damage of intracellular proteins and DNA by way of oxidation and by activating a number of pathways that lead to cell death.[3]

Stroke elicits multiple processes that lead to cell death: excitotoxicity, acidotoxicity and ionic imbalance, peri-infarct depolarization, oxidative and nitrative stress, inflammation and apoptosis.[4]

Stroke symptoms typically start suddenly, over seconds to minutes, and in most cases do not progress further. The symptoms depend on the area of the brain affected.[5] The more extensive the area of brain affected, the more functions that are likely to be lost. Some forms of stroke can cause additional symptoms. For example, in intracranial hemorrhage, the affected area may compress other structures. Most forms of stroke are not associated with headache, apart from subarachnoid hemorrhage and cerebral venous thrombosis and occasionally intracerebral hemorrhage.[6]

Two major approaches have been developed to treat ischemic stroke: recanalization and neuroprotection. At present, Alteplase, recombinant tissue-type plasminogen activator (rt-PA) is the only approved therapy for acute ischemic stroke. Among more than 700 drugs which have been studied and found to be effective in animal stroke models, yet none has been proven efficacious on the basis of a positive phase III trial except a new free-radical tapering agent, NXY-059 .Whereas the goal of using thrombolytics or mechanical devices is the restoration of blood flow, neuroprotective regimen approach is aimed to protect the ischemic penumbra hence to prevent the ischemic core lesion growing.[7]

Various neuroprotective agents interfere with various steps of ischemic cascade. Key agents currently in

development include citicoline (cell membrane stabilizer), traxoprodil (NMDA antagonist), DP-B99 (metal ion chelator) and NXY-059 (a novel free-radical trapping agent). Erythropoietin is thought to act as neuroprotectant by multiple mechanisms in experimental studies. Erythropoietin is the first drug that was found effective in humans in acute ischemic stroke probably because the clinical trial was a "human rat trial".[8] The new free-radical scavenger NXY-059 was tested in animal experiments and positive results could have been translated to humans successfully in a phase III study. It is appealing to develop combination therapies for ischemia that combine vascular and parenchymal effects, especially because preclinical data suggest that many drug combinations improve neuroprotection and extend the therapeutic window in ways not possible with a single agent.[7]

Naturally occurring plant contents such as phenolic compounds, carotenoids, ascorbic acid, thiols, and tocopherols have also shown anti-oxidant activity that include scavenging free radical species, inhibiting the production of reactive species resulting from normal cell metabolism. Thereby prevents the damage to lipids, proteins, nucleic acids and subsequent cellular damage and death.[9]

Stroke

A stroke, sometimes referred to by the older term cerebrovascular accident (CVA), is the rapid loss of brain function due to disturbance in the blood supply to the brain. This can be due to ischemia (lack of blood flow) caused by blockage (thrombosis, arterial embolism), or a hemorrhage. As a result, the affected area of the brain cannot function, which might result in an inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or an inability to see one side of the visual field.

A stroke is a medical emergency and can cause permanent neurological damage and death. Risk factors for stroke include old age, high blood pressure, previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, tobacco smoking and atrial fibrillation. High blood pressure is the most important modifiable risk factor of stroke. It is the second leading cause of death worldwide.[5]

❖ Signs and symptoms

Stroke symptoms typically start suddenly, over seconds to minutes, and in most cases do not progress further. The symptoms depend on the area of the brain affected. The more extensive the area of brain affected, the more functions that are likely to be lost. Some forms of stroke can cause additional symptoms. For example, in intracranial hemorrhage, the affected area may compress other structures. Most forms of stroke are not associated with headache, apart from subarachnoid hemorrhage and cerebral venous thrombosis and occasionally intracerebral hemorrhage.[6]

❖ Classifications

Strokes can be classified into two major categories: ischemic and hemorrhagic. Ischemic strokes are those that are caused by interruption of the blood supply, while hemorrhagic strokes are the ones which result from rupture of a blood vessel or an abnormal vascular structure. About 87% of strokes are caused by ischemia and the remainder by hemorrhage. Some hemorrhages develop inside areas of ischemia "hemorrhagic transformation". It is unknown how many hemorrhages actually start as ischemic stroke.[10]

1. Ischemic

Cerebral infarction and Brain ischemia

In an ischemic stroke, blood supply to part of the brain is decreased, leading to dysfunction of the brain tissue in that area. There are four reasons why this might happen:

- Thrombosis (obstruction of a blood vessel by a blood clot forming locally).
- Embolism (obstruction due to an embolus from elsewhere in the body).
- Systemic hypo perfusion (general decrease in blood supply, e.g., in shock).
- Venous thrombosis.

2. Hemorrhagic

Intracranial hemorrhage is the accumulation of blood anywhere within the skull vault. A distinction is made between intra-axial hemorrhage (blood inside the brain) and extra-axial hemorrhage (blood inside the skull but outside the brain). Intra-axial hemorrhage is due to intra-parenchymal hemorrhage or intraventricular hemorrhage (blood in the ventricular system). The main types of extra-axial hemorrhage are epidural hematoma (bleeding between the Dura mater and the skull), subdural hematoma (in the subdural space) and subarachnoid hemorrhage (between the arachnoids mater and pia mater). Most of the hemorrhagic stroke syndromes have specific symptoms (e.g., headache, previous head injury).[4]

Pathophysiology

a) Cerebral blood flow

Normal CBF is approximately 50 to 60 ml/100g/min. and varies in different parts of the brain. In response to ischemia, the cerebral auto-regulatory mechanism compensate for a reduction in CBF by local vasodilatation, opening of collaterals, and increasing the extraction of oxygen and glucose from the blood. However, when the CBF is reduced to below 20 ml/100g/min, an electrical silence ensues and synaptic activity is greatly diminished in an attempt to preserve energy stores. CBF of less than 10 ml/100g/min results in irreversible neuronal injury.[8]

b) Energy failure

Brain is almost exclusively dependent on the continuous steady flow of glucose and oxygen to undergo oxidative phosphorylation for energy production because it has no stores of energy and deprivation occurs in minutes only.

The first consequence of CBF reduction is the depletion of substrates, particularly oxygen and glucose that causes accumulation of lactate via anaerobic glycolysis. Acidosis may enhance free-radical formation, interfering with intracellular protein synthesis and worsen ischemic brain injury; yet the mechanisms of the deleterious effects of acidosis are still unknown.[11] Energy failure leads to perturbation of the Na^+/K^+-ATPase, and Ca^{2+}/H-ATPase pumps; in addition Na^+-Ca^{2+} transporter is reversed.[12] Subsequent ion dyshomeostasis (elevation of intracellular Na^+, Ca^{2+}, Cl^- and elevation of extracellular K^+ levels) causes to cytotoxic edema and leads to events triggered by excess of intracellular Ca^{2+}.[7]

c) Excitotoxic brain injury

The concept of excitotoxicity, introduced by Olney in 1969, was based on a set of observations that included neuronal injury with local application of glutamate and other acidic amino acids (aspartate, N-methyl-D-aspartate [NMDA], homocysteine, and cysteine). Since this description was published, other excitotoxic mediators have been delineated, including catecholamines (dopamine, norepinephrine), nitric oxide (NO), and related species. Glutamate, the most abundant EAA in the brain, serves a variety of important functions (metabolic, neurotrophic, and neurotransmitter) and is compartmentalized in neurons. The healthy adult brain has the ability to clear extracellular glutamate by rapid uptake; however, under conditions in which energy stores are depleted, such as in hypoxia-ischemia, glutamate efflux into the extracellular compartment due to cellular depolarization, coupled with its impaired uptake, results in increases in intracellular Ca^{2+}. Interference with cysteine uptake (causing depletion of cellular glutathione stores responsible for protecting against oxidative stress) results in neuronal injury. Excitotoxic injury is characterized by its maximal effects on neuronal dendrites and soma, with relative sparing of axons, glia, and ependymal and endothelial cells, possibly due to differences in synaptic input, density, distribution of membrane glutamate receptors, and intrinsic defense mechanisms. Glutamate activates three major families of ionophore-linked receptors (NMDA, amino- 3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA], and kainate) and metabotropic receptors that activate second messenger systems. Although glutamate release simultaneously stimulates NMDA and AMPA receptors, in vitro studies demonstrate that glutamate toxicity occurs in two distinct phases: (1) excitotoxicity is rapidly triggered by brief intense stimulation of NMDA receptors, which is critically dependent on the presence and influx of extracellular Ca^{2+} through the NMDA-gated receptor channel complex; and (2) a slowly triggered process by the prolonged stimulation of AMPA/kainate receptors that have limited Ca^{2+} channels. Metabotropic glutamate receptors modify excitotoxic injury, rather than directly mediate the deleterious process.[13]

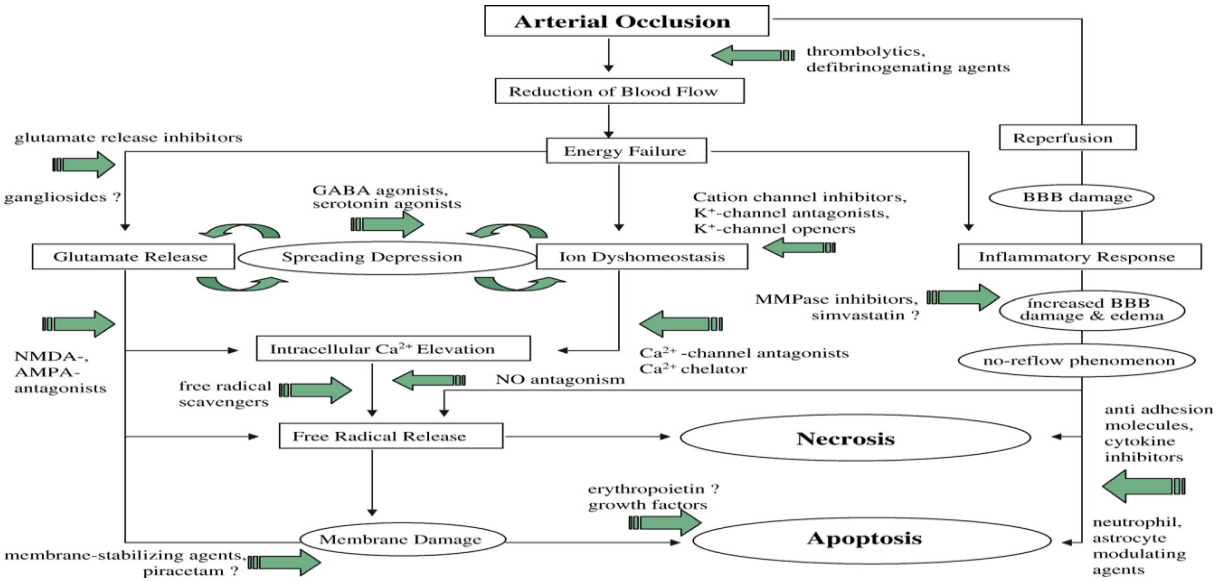

Figure. Pathophysiological changes during cerebral ischemia and reperfusion injury.

d) Role of Ca^{2+} overload

The cascade of events responsible for glutamate excitotoxicity includes three distinct processes: (1) induction, whereby extracellular glutamate efflux is transduced by receptors on the neuronal membrane to cause intracellular Ca^{2+} overload, which leads to lethal intracellular derangements; (2) amplification of the derangement, with an increase in intensity and involvement of other neurons; and (3) expression of cell death triggered by cytotoxic cascades. Excess release of Ca^{2+} and its intracellular influx is thought to be the primary trigger for a variety of complex, deleterious intracellular processes that result from activation of catabolic enzymes such as phospholipases (which lead to cell membrane breakdown, arachidonic acid, and free radical formation) and endonucleases (which lead to fragmentation of genomic DNA and energy failure due to mitochondrial dysfunction). Distinct neuronal populations are selectively vulnerable to excitotoxic injury, possibly from differences in excitatory synaptic

inputs, density of glutamate receptors, or intrinsic defense mechanisms. Perhaps the most compelling evidence for the role of glutamate excitoxicity following focal ischemia and hypoxic-ischemic brain injury from CA is the neuroprotection observed with antiexcitotoxic strategies including NMDA or AMPA-receptor antagonists.[14]

e) Role of Dopamine and Norepinephrine

Acute efflux of dopamine and norepinephrine into the extracellular space following cerebral ischemia possibly plays a role in the propagation of brain injury. Indirect evidence of the importance of their role in cerebral ischemia stems from amelioration of histopathologic injury following attenuation of ischemia-induced surges in extracellular dopamine pharmacologically with barbiturates, isoflurane, and etomidate. Furthermore, several lines of evidence suggest that catecholamine release and metabolism might underlie the selective vulnerability of striatal neurons to an ischemic insult. For example, depletion of catecholamine stores by a-methyl-paratyrosine exerts a strong protective effect on ischemic damage to nerve terminals, and reduction of striatal dopamine content by lesioning the nigrostriatal tract protects intrinsic striatal neurons from injury following global cerebral ischemia. Although the precise mechanism of neuronal injury by dopamine is unclear, by-products of its metabolism, such as hydrogen peroxide, superoxide ion, and hydrogen radicals, have been implicated in this deleterious process.[13]

f) Role of Nitric Oxide

NO, a free radical gas synthesized from the amino acid L-Arginine by the enzyme NO synthase (NOS), is produced by a variety of sources (vascular endothelium, neurons, glia, macrophages, white blood cells). NO has several functions in the brain, including regulation of CBF, neurotransmission, and modification of inflammation. At least three isoforms of NOS have been identified: the constitutively expressed neuronal and endothelial isoforms (encoded on chromosome 12 and 7) and the inducible isoform (encoded on chromosome 17). Neuronal NOS–containing neurons are widespread in the brain, including in the cerebral cortex, hippocampus, and striatum. NO plays a dual role in ischemic neuropathology beneficial, in that it is a potent vasodilator, and cytotoxic, in that it inhibits important enzyme systems such as complexes I and II of the mitochondrial transport chain. Formation of the oxidant perioxynitrite by combination of NO and superoxide anion is considered to be an important trigger in cytotoxicity. Perioxynitrite generation leads to formation of other ROS, hydroxyl free radicals, and nitrogen dioxide, resulting in nitrosylation of tyrosine residues in proteins. One way by which NO is thought to kill neurons is energy-dependent activation of the DNA repair enzyme poly-(ADP-ribose) polymerase, leading to consumption of ATP, nicotinamide, and cell death.[15]

g) Role of ROS

Reactive oxygen species are highly reactive and short-lived molecules that are formed by various pathways. The initial step for ROS production is the univalent reduction of molecular oxygen (O_2) to form superoxide O_2^-. This process is mediated by NADPH oxidase, Xanthine oxidase, or the mitochondrial electron transport chain. A significant amount of electrons are directed to these pathways.

Cerebral vascular endothelial cells have a unique function of forming a barrier which prevents the free flow of ions, polar molecules from blood into brain tissue. Disruption of this barrier during stroke is important because it might lead to local edema due to increased vascular permeability which in turn can decrease the perfusion of the area. Cerebral reperfusion increases the hazardous effect of early ischemic injury by further release of ROS and accumulation of activated neutrophils.

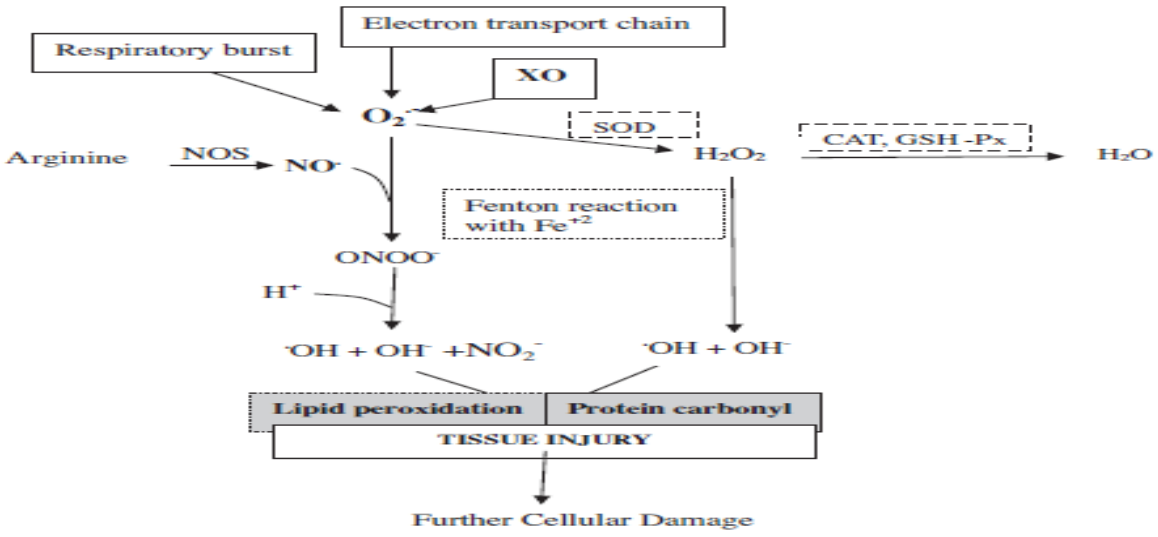

Figure- Production and inhibition of ROS during cerebral ischemia and reperfusion injury.

This cascade of events is known as reperfusion injury. In an animal model, it was shown that ROS are produced as soon as ischemia begins. After a gradual increase, their level reaches a peak for a short period with the beginning of reperfusion and decreases to the control level with. Associated cellular damage is correlated well with the level of ROS. In conjunction with this, increased cerebrovascular permeability was shown to be connected to the initial phase of reperfusion that can be reduced by inhibition of ROS production.[16]

In addition to ROS induced cytotoxicity, other factors participate in I/R injury such as

1. Increased intracellular calcium due to ROS related sarcoplasmic reticulum dysfunction and acidosis,
2. Decreased ATP level and
3. Change in mitochondrial permeability.

h) Inflammation: Ischemic injury triggers inflammatory cascades in the brain parenchyma that may further amplify tissue damage by many mechanisms. Within minutes of occlusion, there occurs upregulation of pro-inflammatory genes which produces mediators of inflammation such as platelet-activating factor, tumor necrosis factor α, and interleukin 1 β. After the expression of adhesion molecules (including intercellular adhesion molecule 1 and selectins) at the vascular endothelium, neutrophils transmigrate from the blood into the brain parenchyma, followed by macrophages and monocytes. Whereas microvascular obstruction by neutrophils (no-reflow phenomenon) can worsen the degree of ischemia, production of toxic mediators by activated inflammatory cells and injured neurons (cytokines, nitric oxide, superoxide and prostanoids) can amplify tissue damage. Neutrophils are the first inflammatory cells to arrive to the ischemic tissue as early as within hours after reperfusion.[17] Macrophages and monocytes arrive within few days. In addition, the inflammatory reaction might also be linked to apoptosis. The pathogenic role of neutrophils and other leukocytes in cerebral ischemia is still a subject of debate[17], microglia and macrophages may even be beneficial through their role in tissue remodeling.[18]

i) Apoptosis

Triggered by a number of processes, including excitotoxicity, free-radical formation (damaging cellular lipids, proteins and nucleic acids), inflammation (activation of FAS-receptor, e.g., through binding of tumor necrosis factor α), mitochondrial and DNA damage, and cytochrome c release from mitochondria, apoptosis occurs after milder ischemic injury, particularly within the ischemic penumbra. Apoptosis is an energy-consuming process, so reperfusion could potentiate apoptosis by restoring cellular energy. Several mediators facilitate cross communication between apoptotic cell death pathways: the calpains, cathepsin B, nitric oxide, and poly-(ADP-ribose) polymerase. Following ischemia, caspase activation occurs in response to pro-apoptotic signals such as down-regulation of Bcl-2 and up-regulation of the Bax/Bid and Death receptor family. Caspase 3 activation may be a downstream event in the apoptotic cascade. Caspases 1, 3, 8, and 9 are involved in cerebral ischemia. Activated caspases are protein-cleaving enzymes and more than 30 proteins can be cleaved that lead to characteristic DNA laddering and cleavage of structural proteins (such as laminin, actin, gelsolin), which are essential for the integrity of the nucleus and the cell. Apoptotic cell morphology differs greatly from necrotic cell morphology. Necrotic cells become edematous and lose their cellular architecture by cytoskeletal breakdown. Apoptotic cell is characterized by a number of morphological features: shrinkage of the cytoplasm, marked condensation of chromatin, membrane-blabbing, and fragmentation of the cell by separation of the protuberances to form multiple small membrane-bound bodies that contain intact organelles and/or dense clumps (apoptotic bodies). Apoptotic cells are rapidly removed by phagocytosis without eliciting an inflammatory reaction. To date there are only few studies demonstrating apoptotic features in human stroke.[19]

Role of Antioxidants: Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidants are constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc.

Antioxidants defense both enzymatic and non-enzymatic reactions protect the body against oxidative damage. Non enzymatic antioxidants are frequently added to the food to prevent lipid oxidation. Several lipid antioxidants can exert pro-oxidant effect towards other molecule under certain circumstances thus the antioxidants for food and therapeutic use must be characterized carefully.[20]

❖**Antioxidants in the prevention and treatment of neurodegenerative disorders:** The distribution of protective antioxidants in the brain has some interesting features. For instance, there is a relatively high concentration of the water-soluble antioxidant vitamin C in the brain. However, vitamin E concentrations are not remarkably different from those in other organs. The concentration of antioxidants varies within the different regions of the brain. For instance, the lowest concentration of vitamin E is found in the cerebellum. It has also been shown that enzymatic antioxidants, such as catalase, are found in lower concentrations in the brain, as compared to other tissues. These facts may also contribute to the potential OS in the brain.[21]

1. ***Vitamin E (a-tocopherol):*** Vitamin E is the most potent antioxidant that can break the propagation of the free radical chain reaction in the lipid part of the biological membrane. Among the antioxidants, it has shown some promise in the treatment of AD.[22] Vitamin

E, along with dietary fats, is absorbed from the intestine and secreted into the circulation in chylomicrons. It is transported in the circulation in plasma lipoprotein. The liver controls vitamin E plasma concentrations through the incorporation of plasma very low-density lipoproteins (VLDL), by the α-tocopherol transfer protein.[23] Genetic defects in this protein cause vitamin E deficiency in humans which in turn lead to peripheral neuropathy due to the resultant impaired lipoprotein delivery of vitamin E to the nervous system. Alpha-tocopherol concentrations in the peripheral nerves are especially sensitive to variations in plasma vitamin E.[24] In rats, it was shown that in the long term, low levels of antioxidants, such as vitamin E, ascorbic acid and GSH in all tissues could lead to tissue peroxidisability. Vitamin E deficiency also influences the activities of SOD, catalase and glutathione peroxidase.[15]

2. ***Vitamin C (ascorbic acid):*** Humans and other primates cannot synthesize this vitamin, whereas most mammals (e.g., rat and mouse) produce it endogenously in the liver.[25] Ascorbic acid is oxidized to dehydroascorbate, which can undergo irreversible hydrolysis to 2,3-diketo- L-gulonic acid, with decarboxylation to CO_2 and components of the pentose phosphate cycle or to oxalic acid plus threonic acid. Vitamin C has a variety of roles, one of which is the regeneration of vitamin E.[26] Vitamin C is found at higher than plasma levels in a variety of tissues, including the brain (there is a greater than 10-fold gradient between the concentration of ascorbic acid in brain and serum). It is believed to be a critical cofactor of dopamine β- hydroxylase, and to be involved in catecholamine biosynthesis. It also inhibits peroxidation of membrane phospholipids, and acts as a scavenger of free radicals.[15]

3. ***Melatonin:*** Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamide secreted by the pineal gland (which is located in the dorsal surface of the hypothalamus) and has structural similarities to serotonin. It is so called because it has the ability in certain fish, reptiles and amphibians to temporarily turn the skin a dark color, by stimulating production of the pigment melanin. Today, melatonin is known as a stringent biological modulator of mood, sleep, retinal physiology, sexual behavior, seasonal- reproductive physiology and behavior, circadian rhythms, and immunological functions. Melatonin is highly lipophilic and, when administered exogenously, can readily cross the BBB to access neurons and glial cells. Moreover, there is experimental evidence that melatonin influences aging and age-related processes and disease states. These roles are apparently related to its potency as a free radical scavenger.[27] Several studies have assessed the neuroprotective effects of melatonin as an antioxidant performed a unilateral lesion of SNpc in rats with the neurotoxin 1-methyl-4-phenylpyridinium (MPP+). He found increased lipid peroxidation (by 117% compared to controls), and decreased tyrosine hydroxylase activity (60% of controls) in the SNpc 4 hours later.[28] Treatment with melatonin, however, led to almost complete recovery of both the lipid peroxidation reduction (99% of control) and tyrosine hydroxylase (TH) levels (96% of control). One week after MPP+ infusion, the untreated rats had a further reduction of tyrosine hydroxylase activity (52% of control). In contrast rats given continuous melatonin treatment (twice a day for 5 days), showed a partial, but not statistically significant, recovery of tyrosine hydroxylase activity (71% of control). Another study showed the potent protective effect of melatonin against oxidative damage induced by kainic acid (KA), a glutamate receptor agonist in rat brain. Kainic acid was directly injected into the unilateral striatum of adult SD rats. The group was administered melatonin (10 mg/kg) *i.p.* 1 h before and 1, 3 and 5 after. Three days later, the control group showed significant apoptotic cortical neuronal death, whereas apoptosis was significantly attenuated in the melatonin treated group. Biochemical studies have indicated that kainic acid can induce OS, as manifested by a decrease in total GSH and GSSG and an increase in the GSSG/GSH ratio in the striatum and cortex compared with the contra-lateral brain regions. In the kainic acid injected striatum, melatonin did not reduce OS, but in adjacent areas, OS was significantly reduced by melatonin.[26] In conclusion, although melatonin can pass the BBB, as shown in animal studies, its use in the treatment of neurodegenerative diseases is limited since the possible effects on other systems have not been fully characterized.[16]

4. ***Reduced glutathione (GSH):*** Reduced glutathione (GSH)-gamma-glutamylcysteinylglycine is a ubiquitous tri-peptide, formed from the amino acids glutamate, glycine, and cysteine by two ATP-dependent enzymatic reactions.[29] The availability of cysteine is critical for the synthesis of GSH in most cells. GSH is a major intracellular antioxidant and its antioxidant activity depends upon the thiol group within the molecules. Intracellular GSH is maintained in its thiol form by glutathione disulfide (GSSG) reductase, which requires NADPH. GSH plays a critical role in detoxification of peroxides and electrophilic toxins as a substrate for GSH peroxidase and GSH-S-transferase. Nearly all the plasma GSH derived from GSH synthesized in the cytosol of hepatocytes and released by carrier- mediated transport. Plasma GSH is cleared by the kidney and other organs (e.g., intestine and lungs) by carrier-mediated transport and breakdown by gamma-glutamyltranspeptidase (GGT) and dipeptidase. Deficiencies of GSH (by buthionine sulfoximine, which inhibits γ-glutamyl transpeptidase, the producing enzyme of GSH), demonstrate the need for cellular protection from endogenous ROS.[30]

5. ***SOD:*** Superoxide dismutase (SOD) is the most widely studied large protein molecule. SOD converts superoxide to hydrogen peroxide (H_2O_2) as follows

$2O^{\bullet -2} + 2H^{+} \rightarrow H_2O_2 + O_2$ (Farber-reaction).

Different forms have been described. One form, containing copper and zinc at its active site, is found in the cytoplasm of cells, and an isoform of this molecule is present in extracellular fluids such as plasma. A third isoform containing manganese at its active site is located in the mitochondria. Trace metals such as copper, zinc and manganese are essential for maintaining the antioxidant activity of SOD. A novel study, performed by[31], showed that augmentation of the natural antioxidant system of wild type worms (*Caenorhabditis elegants)* with Euk 8 and its analog, Euk 134, increased their mean life span by 44%. It was also effective in the treatment of prematurely aging worms, and resulted in the normalization of their life span (a 67% increase). The author concluded that the results supported the theory that ROS is a major determinant of life span and that it can be counteracted by pharmacological intervention.[30]

6. Catalase

Catalase is a member of the peroxidases that contains heme at its active site. Catalases are found in peroxisomes in most tissues and they are believed to cross membranes easily.[32] They reduce hydrogen peroxide ($H_2O_2 \rightarrow H_2O + O2$), which is directly produced by some enzymes (e.g., monoamine, xanthine oxidase), to water and oxygen. The activity of this enzyme is lower in the brain than the liver. Euk-8, a salen-manganese complex, may be regarded as a prototype molecule of a new class of synthetic catalytic scavengers with combined SOD and catalase activity confirmed SOD activity of Euk-8 and other salen-manganese complexes.[33] Catalase activity was demonstrated via its ability to generate oxygen in the presence of hydrogen peroxide. Euk- 8 was shown to be effective in the treatment of neurodegenerative disease in two in vivo models. In the first, mice received intraventricular injections of 6-OHDA and i.p. injections of Euk-8. Extensive damage, measured by binding of [3H] mazindol, a ligand for the dopamine uptake protein, was observed in the brain hemisphere ipsilateral to the site of injection of 6-OHDA at a relatively high dose. Protection by Euk-8 was significant, but only partial. However, in the same mice, a diminished degree of damage was also detected in the contralateral hemisphere. On this side, full protection by Euk- 8 was achieved.[33] Euk-8 has also been found to protect mice against the neurodegenerative effects of MPTP. Mice injected with MPTP, exhibited a substantial loss of dopaminergic neurons, also assessed by [3H] mazindol binding. Oral treatment with Euk-8 (via the drinking water) completely protected mice from MPTP-induced neurotoxicity. These models indicate that, when administered peripherally, Euk-8 can be beneficial in the treatment of 6-OHDA and MPTP toxicity. However, its permeability through the BBB was not measured.[15]

❖Other Classes of Drugs Used

1. N-methyl-D-aspartate–receptor antagonists

Pre- and post-treatment with dextrorphan, an NMDA-receptor antagonist, improves histologic injury in the hippocampus and the cortex in a 4-VO rat model and attenuates the reduction in loss of activity of calcium dependent protein kinases (protein kinase C and calcium-dependent protein kinase II). Although the NMDA-receptor antagonist dizoclipine (MK801) has been shown to provide significant histologic neuroprotection in animal models of global cerebral ischemia, its clinical use in ischemic stroke has been shown to produce significant undesirable side effects (delirium, psychosis, hallucinations, and so forth). Calcium channel antagonists Because Ca^{2+} is the final common pathway in excitotoxic neuronal injury, nimodipine, a blocker of Ca^{2+} influx, has been studied in the experimental paradigm of global cerebral ischemia. Subcutaneous administration of nimodipine failed to demonstrate any histologic or functional neurologic improvement in the 4-VO rat model of global cerebral ischemia; however, in a rabbit model, intravenous treatment with nimodipine reduced time of EEG recovery and attenuated the decrement in extracellular Ca^{2+} and disruption of the BBB. In this study, arterial blood pressure was maintained at 100 mm Hg following the ischemic insult, thereby offsetting the detrimental hypotensive effects of nimodipine. A prospective, randomized, double-blinded trial with nimodipine in patients who had out-of-hospital ventricular fibrillation failed to demonstrate any improvement in 1-year survival rate; however, it demonstrated some benefit in patients who had delayed resuscitation (10 minutes).[7]

2. g-Aminobutyric acid and g-aminobutyric acid agonists

The premise of using g-aminobutyric acid (GABA) or its agonists as neuroprotectants is based on their inhibitory properties by way of opening of the Cl^- channels. Pretreatment with GABA attenuated histologic injury and improved neurobehavior in a gerbil model of global cerebral ischemia. Treatment following the insult failed to demonstrate any improvement in these parameters. Chlormethiazole, a GABA agonist with anticonvulsant, hypnotic, and sedative properties, failed to demonstrate any improvement in histologic injury or neurobehavior in a rat model of global cerebral ischemia. Furthermore, local infusion of chlormethiazole by way of microdialysis did not alter ischemia-evoked release of dopamine, serotonin, or their metabolites in the ischemic striatum. Intraperitoneal administration of g-hydroxybutyrate improved histologic injury and neurobehavioral outcomes in a 4-VO rat model of global cerebral ischemia. Tiagabine, a selective inhibitor of GABA reuptake, failed to demonstrate any improvement in histologic outcome in the gerbil model when given as a pretreatment.[6]

3. Anticonvulsants

The basis for the use of anticonvulsants in ischemic neuroprotection is their ability to stabilize neurons by way of hyperpolarization of the membrane potential by blocking voltage-gated Na^+ channels. Treatment with phenytoin attenuates accumulation of K^+ in cerebrospinal

fluid in animals subjected to circulatory arrest. Some studies of phenytoin treatment have demonstrated attenuation of brain edema, increased Na^+/K^+-ATPase activity, decreased intracellular Na^+ concentration, and attenuated accumulation of lactate and free fatty acids. Lamotrigine attenuates ischemia induced increases in extracellular glutamate levels and improves histologic outcomes in the gerbil and rat models of global cerebral ischemia.[35]

4. Magnesium

Magnesium sulfate has multimodal actions. It is an NMDA receptor, a calcium antagonist, and a vasodilator. In a rat model of global cerebral ischemia, pre- and post-treatment with bolus intravenous magnesium sulfate followed by a continuous intravenous infusion attenuated injury to CA1 hippocampal neurons. Other investigators have reported that neuroprotection is demonstrated only when magnesium sulfate is administered in combination with mild hypothermia.[35]

5. Anesthetic agents

Barbiturates have long been known to exert neuroprotection by coupled decreases in cerebral metabolic rate of oxygen (CMRO2) with CBF and by inhibiting agonist-induced cerebral vasoconstrictor responses, protein kinase C, ischemia-induced increases in free fatty acids, and EAA release. In experimental global cerebral ischemia, however, results have been disappointing. Treatment with pentobarbital failed to improve survival in a dog model of global cerebral ischemia.[36] Clinical investigation with barbiturates has also proved to be disappointing, with lack of therapeutic benefit in the hypoxic-ischemic brain injury from CA and near-drowning. Inhalational anesthetics (halothane, isoflurane) have not been thoroughly investigated in the setting of global cerebral ischemia. The premise for their use is their ability to attenuate cerebral metabolic demand, enhance regional CBF, attenuate ischemia-evoked EAA and catecholamine efflux, and attenuate calcium influxes.[13]

6. *Cyclooxygenase inhibitors*

Nimesulide, a cyclooxygenase-2 inhibitor, attenuated injury to the CA1 region of the hippocampus in a gerbil model when administered orally or intraperitoneally as a pre- or post-treatment (up to 24 hours). Further experimental studies in other animal models are needed to confirm these findings and bring these agents into the clinical paradigm.[37]

7. Immunosuppressants

Tacrolimus (FK506) and cyclosporine are immunophilin and calcineurin inhibitors that attenuate apoptotic cell death. Chronic administration of both agents (for 3 days) before the ischemic insult demonstrated neuroprotection in the CA1 region at 7 days of reperfusion in a rat model of global cerebral ischemia.[38] These agents also attenuated calcineurin activity in the CA1, CA3, and dentate gyrus regions of the hippocampus up to 24 hours following the ischemic insult. Pretreatment with cyclosporin and FK506 inhibits dephosphorylation of the proapoptotic protein Bad. The inability of cyclosporine to cross an intact BBB is a significant therapeutic concern. These agents require more rigorous testing with treatment in the postischemia paradigm across different species and animal models of global cerebral ischemia.[39]

CONCLUSION

In this review, we have discussed every possible biochemical marker which can possibly take part in stroke situation. In addition to it we have given a complete incite about all the possible treatments which we can use to overcome the stroke situation.

REFERENCES

1. W. Dalton Dietrich. Neurobiology of Stroke. International review of Neurobiology, 1998; 42.
2. Kaplan B., Brint S., Tanabe J. Temporal thresholds for neocortical infarction in rats subjected to irreversible focal cerebral ischemia. Stroke, 1991; 22: 1032–39.
3. Rosamond W., Flegal K. Heart disease and stroke statisticse 2007 Update. A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation, 2007; 115: 69-171.
4. Gonzalez R., Hirsch J. Acute Ischemic Stroke. Imaging and Intervention. Springer 2006.
5. Doyle K.P., Simon R.P. Mechanisms of ischemic brain damage. Neuropharmacology, 2008; 55: 310-318.
6. Smith WS. Pathophysiology of Focal Cerebral Ischemia: a Therapeutic Perspective. Journal of Vascular and Intervention Radiology, 2004; 15: S3–S12.
7. Durukan A., Tatlisumak T. Acute ischemic stroke: Overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacology, Biochemistry and Behavior, 2007; 87: 179–197.
8. Sadamoto Y, Igase K, Sakanaka M, Sato K, Otsuka H, Sakaki S, et al. Erythropoietin prevents place navigation disability and cortical infarction in rats with permanent occlusion of the middle cerebral artery. Biochemical and Biophysical Research Communication, 1998; 253: 26–32.
9. Shri R, Bora KS. Neuroprotective effect of methanolic extracts of *Allium cepa* on ischemia and reperfusion-induced cerebral injury. Fitoterapia 2008; 79: 86-96.
10. Raichle ME. The pathophysiology of brain ischemia. Annals of Neurology, 1983; 13: 2-10.
11. Siesjo BK, Katsura K, Kristian T. Acidosis-related damage. Advances in Neurology, 1996; 71: 209–33.
12. Phan TG, Wright PM, Markus R, Howells DW, Davis SM, Donnan GA. Salvaging the ischaemic penumbra: more than just reperfusion? Clinical and Experimental Pharmacology and Physiology, 2002;

29: 1–10.
13. Harukuni I, Bhardwaj A. Mechanisms of brain injury after global cerebral ischemia. Neurologic Clinics, 2006; 24: 1–21.
14. Atlante A, Calissano P, Bobba A, Giannattasio S, Marra E, Passarella S. Glutamate neurotoxicity, oxidative stress and mitochondria. Federation of European Biochemical Societies Letters, 2001; 497: 1-5.
15. Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. Neuropharmacology, 2001; 40: 959–975.
16. Olmez I, Ozyurt H. Reactive oxygen species and ischemic cerebrovascular disease. Neurochemistry International, 2012; 60: 208–212.
17. Emerich DF, Dean III RL, Bartus RT. The role of leukocytes following cerebral ischemia: pathogenic variable or bystander reaction to emerging infarct? Experimental Neurology, 2002; 173: 168–81.
18. Danton GH, Dietrich WD. Inflammatory mechanisms after ischemia and stroke. Journal of Neuropathology and Experimental Neurology, 2003; 62: 127–36.
19. Bramlett HM, W. Dalton Dietrich. Pathophysiology of cerebral ischemia and brain trauma: Similarities and differences. Journal of Cerebral Blood Flow & Metabolism, 2004; 24: 133–150.
20. Ursini F, Maiorino M, Valente M, Ferr L and Gregolin, C, Biochemica et Biophysica Acta, 1982; 710: 197- 211.
21. Kumar S. Free radicals and antioxidants: human and food system. Advances in applied science research, 2011; 2(1): 129-135.
22. Vatassery GT. Vitamin E: Neurochemistry and implication for Parkinson's disease. Annals of the New York Academy of Sciences, 1992; 669: 92–110.
23. Traber MG, Sies H. Vitamin E in humans. Demand and delivery. Annual Review of Nutrition, 1996; 16: 321–347.
24. Pillai SR, Traber MG, Steiss JE, Kayden HJ. Depletion of adipose tissue and peripheral nerve α-tocopheral in adult dogs. Lipids, 1993; 28: 1095–1099.
25. Chatterjee IB, Majunder AL, Nandi BK, Subramanian N. Synthesis and some major functions of vitamin C in animals. Annals of the New York Academy of Sciences, 1975; 258; 24–47.
26. Chan AC. Partners in defense Vitamin E and vitamin C. Canadian Journal of Physiology and Pharmacology, 1993; 71(9): 725–731.
27. Beyer CE, Steketee JD, Saphier D. Antioxidant properties of melatonin-an emerging mystery. Biochemical Pharmacology, 1998; 56: 1265–1272.
28. Jin BK, Shin DY, Jeong MY, Gwang MR, Baik HW, Yoon KS, Cho YH, Joo WS, Kim YS, Baik HH. Melatonin protects nigral dopaminergic neurons from 1-methyl-4-phenylpyridinium (MPP+) neurotoxicity in rats. Neuroscience Letters, 1998; 245: 61–64.
29. Chen ST. The antioxidant melatonin reduces cortical neuronal death after intrastriatal injection of haingate in the rat. Experimental Brain Research, 1999; 124: 241–247.
30. Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D. Antioxidant Therapy in Acute Central Nervous System Injury: Current State. The American Society for Pharmacology and Experimental Therapeutics, 2002; 54: 271–284.
31. Melov S, Ravenscroft J, Malik S. Extension of life span with Superoxide Dismutase/Catalase Mimetics. Science, 2000; 289: 1567–1569.
32. Halliwell B. and Gutteridge, J. M. C. Free Radicals in Biology and Medicine. Oxford: Oxford University Press, 1999.
33. Baudry M, Etienne S, Bruce A, Puluchi M, Jacobsen E, Malfroy B. Salen manganese complexes are superoxide dismutase mimics. Biochemical and Biophysical Research Communications, 1993; 192(2): 964–968.
34. Smith WS. Pathophysiology of Focal Cerebral Ischemia: a Therapeutic Perspective. Journal of Vascular Intervention of Radiology, 2004; 15: S3–S12.
35. Bentley P, Sharma P. Pharmacological treatment of ischemic stroke. Pharmacology & Therapeutics, 2005; 108: 334-352.
36. Steen PA, Milde JH, Michenfelder JD. No barbiturate protection in a dog model of complete cerebral ischemia. Annals of Neurology, 1979; 5: 343–9.
37. Eduardo CJ, Noël HM, Armando GF, Michel GC, Eduardo M, Olga SL, Bernd LF. Effects of the cyclooxygenase-2 inhibitor nimesulide on cerebral infarction and neurological deficits induced by permanent middle cerebral artery occlusion in the rat. Journal of Neuro-inflammation, 2005; 10.1186: 1742-2094.
38. Uchino H, Minamikawa-Tachino R, Kristian T. Differential neuroprotection by cyclosporine A and FK506 following ischemia corresponds with differing abilities to inhibit calcineurin and the mitochondrial permeability transition. Neurobiological Disorders, 2002; 10: 219–33.
39. Weigl M, Tenze G, Steinlechner B. Asystematic review of currently available pharmacological neuroprotective agents as a sole intervention before anticipated or induced cardiac arrest. Resuscitation, 2005; 65: 21–39.