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CEREBRAL ISCHEMIC STROKE: SEQUELS OF CASCADE

ASHU AGGARWAL *[1], PARVEEN AGGARWAL[2], MAMTA KHATAK[1] AND SUNIL KHATAK[3]

[1] Ram Gopal College of Pharmacy, Sultanpur, Gurgaon, (Haryana, India)
[2] Panjab University Chandigarh, India
[3] Raj Kumar Goel Institute of Technology, 5 kilometer Milestone, Delhi Meerut Road, Ghaziabad, (Uttar Pradesh, India)

**** Corresponding Author*** singlashu@gmail.com

ABSTRACT

Cerebral Ischemia (stroke) is one of the foremost causes of high morbidity and mortality for both developed and developing countries. Cerebral ischemia impairs the normal neurological functions which are triggered by a complex series of biochemical and molecular mechanism. Understanding of mechanisms of injury and neuroprotection in this disease is important to learn new target sites to treat ischemia. In this article, there is clear understanding of ischemic cascade followed by the mechanism of all damaging factors like energy failure, excitotoxicity, oxidative stress, neuroinflammation, cell death modes: necrosis, apoptosis along with histological changes. Further it also discloses the different epidemiology based on the age, gender and races along with current status of the prevalence in India in comparison with western world. The present authors also describe and relate aponecrosis and necroptosis with cerebral ischemia. The main emphasis is given and described along with diagrammatic view.

KEY WORDS

Cerebral Ischemia, Pathophysiology, Epidemiology, Necroptosis, Aponecrosis

ABBREVIATIONS

AIF: Apoptosis Inducing Factor; **AMPA:** α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; **APAF-1**: Apoptotic Proteases-Activating Factor – 1; **ATP**: Adenosine Try Phosphate; **CINC:** Cytokine-induced neutrophil chemoattractant; **DD:** Death Domains; **Diablo:** Direct IAP-Binding Protein with Low pI; **DISC:** Death Inducing Signaling Complex; **DRs:** Death Receptors; **FADD:** Fas-Associated Death Domain; **IAPs:** Inhibitors of Apoptotic Proteins; **ILs:** Interleukins; **LTD:** long term depression; **LTP:** Long Term Potentiation; **MCAO:** Middle Cerebral Artery Occlusion; **MCP-1:** Monocyte chemoattractant protein–1; **MPT:** Mitochondrial Permeability Transition**; Nec-1**: Necrostatin-1; **NF-κB:** Nuclear Factor kappa B; **NMDA:** N-methyl-D-Aspartic Acid; **nNOS:** Neuronal Nitric Oxide Synthase; **PARP:** Poly ADP Ribose polymerase; **PMN:** Polymorphonuclear; **RIP:** Receptor interacting proteins; **ROS:** Reactive Oxygen Species; **Smac:** Second Mitochondrial-Derived Activator of Caspases; **SOD:**

Superoxide Dismutase; **TGF:** Tumor Growth Factor; **TNF**: Tumor Necrosis Factor; **TRADD:** TNF-receptor Associated Death Domain.

INTRODUCTION

Stroke is a major cause of death and long term disability across the globe. WHO defined stroke as 'rapidly developed clinical signs of focal disturbance of cerebral function, lasting more than 24 hrs or leading to death, with no apparent cause other than vascular origin' [1,2]. Cerebral stroke, besides fatality, is the most common cause of disability leading to dependency, crucial both from economic and humanitarian point of view. In the western world stroke is the third leading cause of death next to heart diseases and cancer[3]. From the early 1970s to early 1990s, the estimated number of noninstitutionalized stroke survivors increased from 1.5 million to 2.4 million [4]. Around total number of 1 million cases has been reported in the India related to stroke. The changing pattern of disease occurring in India due to efforts in control of communicable disease have brought in a sharp focus, stroke as one of the major health problems. Around 12% of all strokes occur in population below 40 years [5].

Cerebral ischemic stroke is a neurological disease where neuronal cell death is caused by a serial pathophysiological events, so called 'ischemic cascade' like energy failure, excitotoxicity, oxidative stress, inflammation, apoptosis etc. These all damaging factors are triggered by decreased/blocked blood flow [6,7]. The authors review the progression, understanding and mechanism at cellular levels of all the above devastating events which are results of declined blood supply that leads to neuronal cell death in cerebral ischemic stroke. The present article further deals with epidemiology (global vis-à-vis Indian) and the pathophysiological aspects of ischemic stroke.

WHAT IS CEREBRAL STROKE?

Stroke occurs due to sudden interruption of blood supply (normally caused by a thrombus or embolus occlusion or hemorrhage due to rupture of blood vessel) to a part of brain results in disruption of neurologic functioning. In stroke, the oxygen supply to the brain gets impaired which finally leads to death of neuronal cells [8,9].

For the first time in 1847, Rudolf Virchow introduced the concept that systemic emboli lodging in the cerebrovasculature and vascular occlusion cause stroke, but the therapeutic implications of this notion were not fully implemented until more than a century later [10,11,12].

SYMPTOMS

Symptoms of stroke includes vertigo, sensory loss, nystagmus, anopia, facial numbness, ataxia, disphagia, dysarthria, ophthalmoplegia, hemiparesis, arm & leg paralysis, amnesia, color amnesia, abulia, alexia, urinary incontinence, or coma, depending on arterial territory involved. Major disability is loss of ability to communicate, ambulate, co-ordinate and reason. Many risk factors are identified as the probable cause for ischemia. Several factors may play role in the development of stroke such as environmental factors (e.g. smoking, alcohol consumption, oral contraceptives, diet etc.), comorbidities (e.g. hypertension, coronary heart diseases, atrial fibrillation, aneurysm, asteriovenous malformation, atherosclerosis, diabetes mellitus, etc.) and genetic factors (e.g. age, race etc.). [13,14]

FACTORS

The non-modifiable factors include age, gender, positive family history, ethnicity, previous transient ischemic attack or stroke whereas the modifiable factors include hypertension, diabetes, smoking, lipid disorders – hypercholesterolemia, alcohol intoxication and physical inactivity [15].

CLASSIFICATION OF CEREBRAL STROKE

Stroke basically classified in to two categories; Occlusive and hemorrhagic

Occlusive or ischemic stroke in which the disrupted blood supply is caused by a blocked blood vessel. This results in formation of an embolus or thrombus that occludes an artery. In thrombotic stroke, a blood clot (thrombus) forms inside an artery such as internal carotid artery, proximal and intracranial vertebral arteries or basilar artery, produces lacunes, small infarcts to typical locations include basal ganglia, thalamus, internal capsule, pons and cerebellum. Embolic stroke occurs when a clot breaks, loose and is carried by the blood stream and gets wedged in medium-sized branching arteries [16,17].

Hemorrhage in which the disrupted blood supply is caused by rupture of an extracerebral artery. Eighty percent of stroke are ischemic and include thrombotic and embolic stroke [18,19].

RISK FACTORS

Advanced age (>65 years old), Family history, Male gender, African American, Hypertension, Diabetes, Smoking, Cerebral amyloidosis, Coagulopathies, Anticoagulant therapy, Thrombolytic therapy for acute myocardial infarction (MI) and acute ischemic stroke (can iatrogenically cause a hemorrhagic stroke), Drug abuse (cocaine or other sympathomimetic drugs), Bleeding due to a brain tumor, Atherosclerosis, Heart disease (atrial fibrillation, coronary artery disease, dilated cardiomyopathy, left ventricular hypertrophy), Hyperlipidemia, Hyperhomocysteinemia, Birth control pills, Hyperviscosity (polycythemia, dehydration, sickle cell anemia), Prior transient ischemic attack (TIA), Heavy alcohol consumption, Vascular malformations (aneurysms), Pregnancy/childbirth, Menopause [20,21,22,23,24].

COMPLICATIONS

Stroke complications can include sleep problems, confusion, depression, incontinence, atelectasis, pneumonia, and swallowing dysfunction, which can lead to aspiration, dehydration, or under nutrition. Immobility can lead to thromboembolic disease, deconditioning, sarcopenia, UTIs, pressure ulcers, and contractures. Daily functioning (including the ability to walk, see, feel, remember, think, and speak) may be decreased [25,26].

EPIDEMIOLOGY

Worldwide Incidence In 80% of the cases Ischemic attacks results from atherosclerotic cerebral thrombotic events. Risk of stroke in the first year following ischemic attacks is about 10%. On average, every 45 seconds someone in the United States has a stroke. In the US, ischemic attacks affects at least 200,000 to 500,00 persons per year Population-based studies have reported little change in ischemic attacks incidence during the past few decades, suggesting that the prevalence of atherosclerosis, the most common mechanism of ischemic attacks, has not changed [27,28].

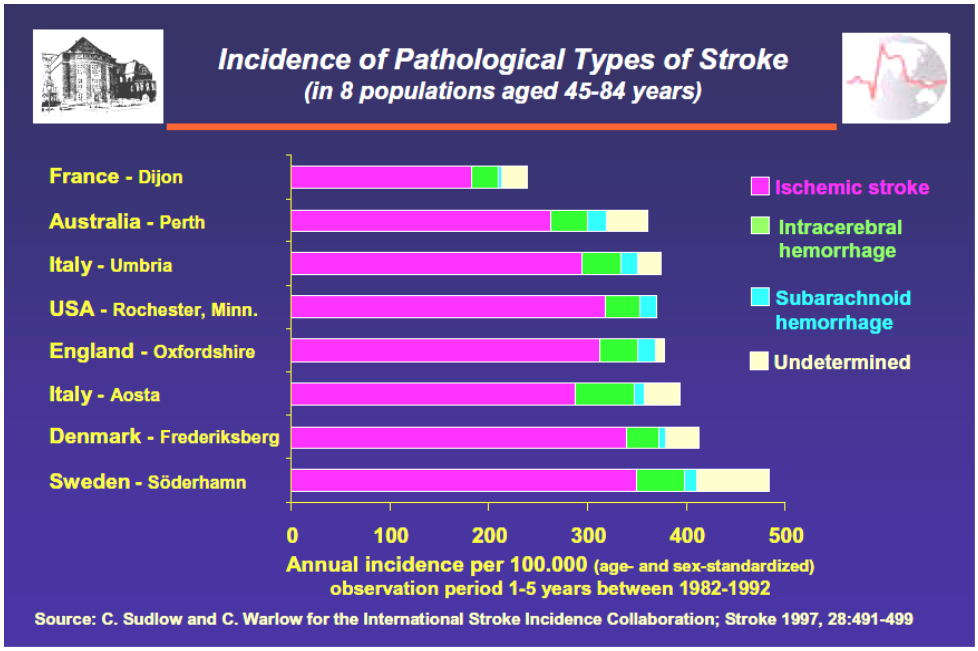

Figure 1.
Global Prevalence of Different Kinds of Cerebral Stroke.

Mortality Stroke accounted for about one of every 15 deaths in the United States in 2003. About 50 percent of these deaths occurred out of hospital. Stroke as an underlying or contributing cause of death about 273,000. On average, about every three minutes someone dies of a stroke. 8 to 12 percent of ischemic strokes and 37 to 38 percent of hemorrhagic strokes result in death within 30 days. From 1993–2003, the stroke death rate fell 18.5 percent, and the actual number of stroke deaths declined 0.7 percent. The 2003 overall death rate for stroke was 54.3. Death rates were 51.9 for white males and 78.8 for black males; and 50.5 for white females and 69.1 for black females. Because women live longer than men, more women than men die of stroke each year. Women accounted for 61.0 percent of U.S. stroke deaths in 2003 [29,30,31].

Age and Gender Each year about 46,000 more women than men have a stroke. Men's stroke incidence rates are 1.25 times greater than women's. The difference in incidence rates between the sexes is somewhat larger at younger ages but nonexistent at older ages. The male/ female incidence was 1.59 for ages 65–69; 1.46 for ages 70–74; 1.35 for ages 75–79 and 0.74 for age 80 and older. In a large, prospective, population-based study, the average age at first presentation with the ischemic attacks was 74 years; men with new Ischemic attacks were significantly younger than were women (mean age, 71 vs. 76 years, respectively)[32,33].

Race Blacks have almost twice the risk of first-ever stroke compared with whites. The age-adjusted stroke incidence rates (per 100,000) for first-ever strokes are 167 for white males, 138 for white females, 323 for black males and 260 for black females. Whites are twice as likely as blacks to have extracranial lesions. Prevalence of intracranial lesions is similar in both groups [34].

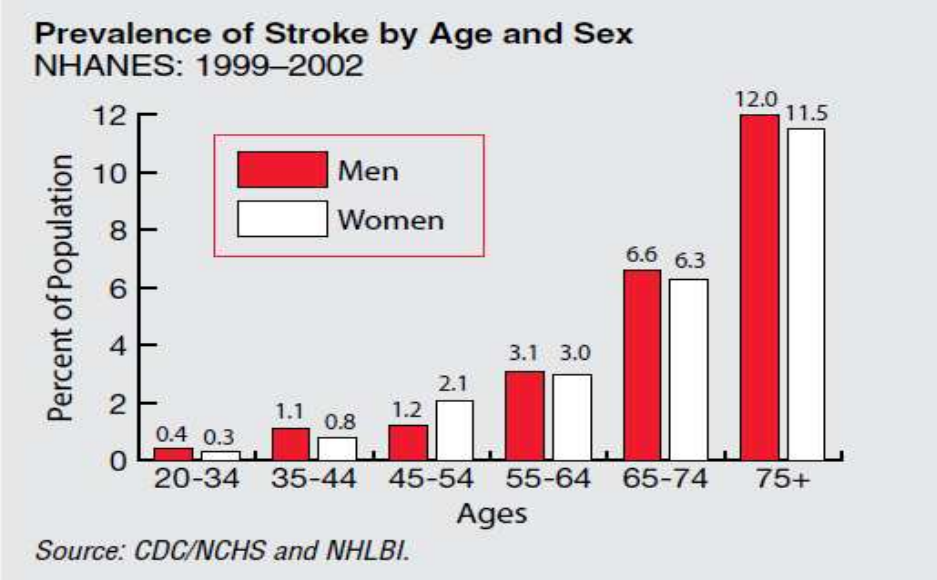

Figure 2.

Epidemiology of Cerebral Ischemia Based On Age and Gender.

INDIAN SCENARIO

Incidence There is a need to take stern steps to collect data on morbidity and mortality due to stroke because the India is ranked among the countries where the information on stroke is minimal. Several population-based surveys on stroke were conducted from different parts of India. During the last decade, the age-adjusted prevalence rate of stroke was between 250-350/100,000. Recent studies showed that the age-adjusted annual incidence rate was 105/100,000 in the urban community of Kolkata and 262/100,000 in a rural community of Bengal. The ratio of cerebral infarct to hemorrhage was 2.21. Hypertension was the most important risk factor. Stroke represented 1.2% of total deaths in India. A comprehensive 5-year prospective study on stroke is currently under way in the city of Kolkata where both the stroke survivors and the stroke death cases are being captured giving the true estimate of stroke incidence rates [35,36].

Mortality Rate There were limited data available on stroke related mortality in India. Although medical certification of the cause of death is a legal requirement, only 13.5% of all deaths in India were medically certified in 1994. Therefore ascertainment of the cause of death was grossly inadequate in India. However, it was estimated that stroke represented 1.2 % of the total deaths in the country, when all ages were included. The proportion of stroke death increased with age, and in the oldest group (> 70 years of age) stroke contributed to 2.4% of all deaths. The gender ratio of death due to stroke was 1.24 One would expect a high mortality of stroke with low prevalence and median annual incidence of stroke in India [37,38].

THE WHOLE CASCADE OF ISCHEMIC BRAIN INJURY

The process of reduction of blood and glucose supply produces brain injury via a variety of cellular and molecular mechanisms that impair the energetic required to maintain ionic gradients[39.40]. The mechanisms involve a complex series of pathophysiological events that are dependent on the severity, duration, and location of the ischemia within the brain.

Within minutes of vascular occlusion, brain tissue is deprived of glucose and oxygen and the acidic by-products start accumulating [41,42]. This loss of nutrients and decrease in pH levels lead to cessation of the electron transport chain activity within mitochondria resulting in a rapid decline in ATP concentration [43,44]. This failure of energy homeostasis is the first event that occurs in stroke. Due to ATP loss, it causes disruption of ionic pumps systems like Na^+-K^+-ATPase, Ca^{2+}-H^+ ATPase, reversal of Na^+-Ca^{2+} transporter resulting in increase in intracellular Na^+, Ca^{2+}, Cl^- concentration and efflux of K^+ [45,46]. This redistribution of ions across plasma membrane causes neuronal depolarization, leading to excess release of neurotransmitters, in general and glutamate in particular that causes neuronal excitotoxicity [47,48]. Glutamate causes excessive increase in Ca^{2+} concentration into nerve cells through overactivation of their receptors which then triggers a variety of processes that can lead to necrosis and apoptosis [49,50]. The processes include Ca^{2+} overload of mitochondria, oxygen free radical formation and activation of caspases-9,3,8, BAD, BAX, & calpains resulting in oxidative stress and apoptosis respectively [51,52].

Ca^{2+} dependent activation of nNOS (neuronal nitric oxide synthase), leading to increased NO production and formation of toxic peroxynitrite ($ONOO^-$) contributes to oxidative stress and excitotoxicity [53,54,55]. Also upregulation of a variety of enzyme systems such as lipases, proteases, phosphatases, kinases and endonucleases activate various inflammatory molecules like cytokines and interleukins (ILs) [56,57] such as TNF-α, NF-κB that results in neuroinflammation [58]. As there is excessive influx of Na^+ and Ca^{2+} & efflux of K^+ and recruitment of inflammatory mediators like leukocytes [59,60] & adhesion molecules, it causes fluid accumulation at injury site resulting in edema formation [61,62]. All these damaging factors lead to irreversible final event in cerebral ischemic stroke i.e. the death of neuron cells and also irreversible loss of neurological function including cognitive functions [63,64] . The mechanism of ischemic stroke development is summarized below (Figure 3).

The ischemic injury depends on the intensity of ischemic insult. The major events that follow during ischemia: energy failure, glutamate mediated excitotoxicity, generation of free radicals (oxidative stress), neurovascular pathophysiology & inflammation, cell death mode: necrosis, apoptosis and neuromodulation are discussed in detail in the text below. Each of the above processes has a distinct time frame, some occurring over minutes, others over hours and days. These processes share overlapping and redundant features.

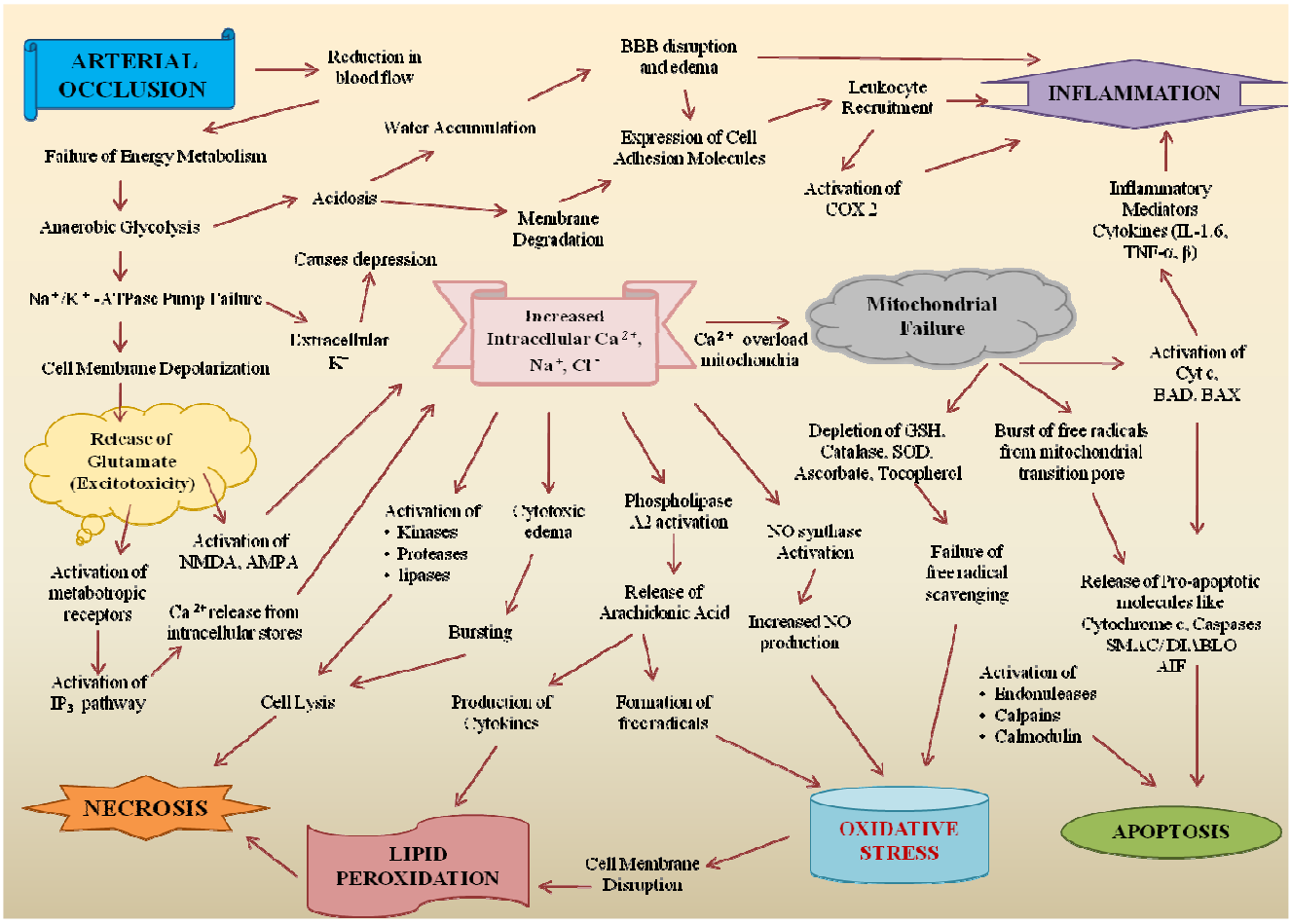

Figure 3.
Pictorial Representation of Cerebral Ischemic Cascade.

Cerebral Ischemia and Energy Failure

The maintenance of cellular energy reserves is vital for cellular survival. The brain has low levels of stored glycogen and is highly dependent on oxidative metabolism [65,66]. The coenzyme NAD^+, a parent compound to NADH, NADP, and NADPH, is an important contributor to ATP production. Cellular NAD^+ holds a key position in the control of fundamental cell processes as it is the major donor of electrons for mitochondrial electron transport to power oxidative phosphorylation. Mitochondrial NAD^+ gets rapidly depleted during ischemia; and also ATP levels become lowered [67,68]. This decreased ATP, reduced NAD^+ stimulate mitochondrial permeability transition (MPT), means mitochondria becomes freely permeable to low MW solutes. The transition causes mitochondrial depolarization, uncoupling and inhibition of oxidative phosphorylation with stimulation of mitochondrial ROS generation, mitochondrial swelling [69,70], and release of intramitochondrial solutes into the cytosol such as cytochrome-c, second mitochondrial-derived activator of caspases (SMAC), direct IAP-binding protein with low pI (DIABLO), apoptosis inducing factor (AIF). MPT is an attractive hypothesis because it takes into account the multiple injury mechanisms are known to be activated during ischemia and reperfusion [71,72].

Cerebral Ischemia and Excitotoxicity

Excitotoxicity, the term coined by Olney in 1969, occurs due to excess release of excitatory amino acid glutamate and excessive activation of their receptors. During acute and chronic ischemia, disruption of energy

metabolism impairs the clearance of glutamate due to transporter dysfunction. Also ATP depletion causes neuronal membrane depolarization, which opens voltage-gated Ca^{2+} and Na^{+} channels and releases excitatory glutamate [73,74,75]. Under ischemic conditions, glutamate is massively released (initially mediated by vesicular release from nerve terminals, and later by reverse transport from astrocytes). Unfortunately, such concentrations of glutamate are neurotoxic. Excess Glutamate release causes overactivation of NMDA, AMPA and kainate receptors and results into excessive influx of Ca^{2+}, Na^{+}. Ca^{2+} overloads mitochondria results in free radical production, activation of inflammatory mediators that contributes to neuronal injury and cell death [76,77,78]. Marked neuronal cell body swelling and dendrite swelling occur, hallmarks of necrosis death, as Na^{+} and Ca^{2+} entry is joined by the influx of Cl^{-} and water [79,80,81,82].

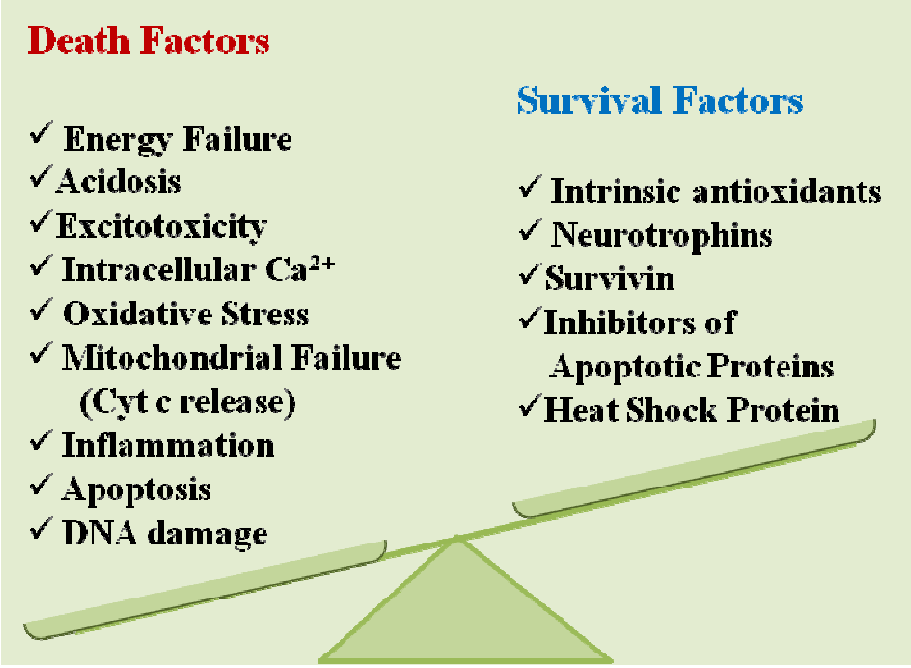

Figure 4.

Dominance of Death Factors over Survival Factors, Activated during Cerebral Ischemic Stroke: During ischemic insult, both death as well as cell survival components get activated but among them death factors takes over survival factors. Therefore leads to neuronal cell death.

Cerebral Ischemia and Oxidative Stress

Normally oxidative stress is being caused by the imbalance between free radical production and degradation. Brain is most susceptible to oxidative stress due to large consumption of oxygen [83,84,85]. Natural formation of oxidants during mitochondrial electron transport, auto-oxidation of some neurotransmitters (e.g. norepinephrine, dopamine) and in ischemic attacks of events during ischemia can result in oxidant formation and subsequent tissue damage [86,87]. Superoxide and nitric oxide have important roles in health, serving as regulators of blood flow and neurotransmission. Oxidative stress can be traced primarily to formation of these molecules. Pathologic consequences results due to alteration in the activities of these molecules [88,89].

Leakage during mitochondrial electron transport, altered mitochondrial metabolism and inflammatory responses to injury leads to superoxide production [90,91,92]. Defense mechanism of Brain against superoxide includes dietary free-radical scavengers (ascorbate, α-tocopherol), the endogenous tripeptide glutathione, and enzymatic antioxidants. Enzymatic antioxidants regulate

superoxide concentration by dismutation of superoxide to hydrogen peroxide (superoxide dismutase or SOD), which is then converted to water (peroxidases such as glutathione peroxidase and peroxiredoxin) or dismuted to water and oxygen (Catalase) [93,94,95]. Ischemia can also induce increased expression of these enzymes. In ischemia endogenous antioxidant capacity can be overwhelmed, leading to increased superoxide and hydrogen peroxide concentrations [96,97].

Production of nitric oxide formation is both constitutive and inducible during ischemia [98,99,100]. Overproduction of Ischemia-induced nitric oxide can also be caused by glutamatergic-mediated increases in intracellular calcium concentration, resulting in a calmodulin-dependent upregulation of nitric oxide synthase (NOS) [101,102]. Nitric oxide can be consumed by reacting with hemoglobin. Flavohemoglobin-based enzymes (nitric oxide reductase, nitric oxide dioxygenase) capable of specifically metabolizing nitric oxide have been identified in mammalian cells. Reaction with superoxide yielding peroxynitrite is a well known non-enzymatic mechanism which regulates nitric oxide concentration [103,104].

In accordance with excessive nitric oxide production, nitrosative damage can also aggravate *via* independent nitrosylation of protein heme sites (e.g. cytochrome *c*) or through its reaction products with oxygen or other nitrogen oxides [105,106,107]. Superoxide can cause oxidative damage of iron/sulfur clusters of aconitase, an important enzyme in the tricarboxylic acid cycle [108,109]. Superoxide can also participate in the peroxynitrite formation and can be involved in the iron-catalyzed Haber–Weiss reaction which causes the conversion of hydrogen peroxide to be hydroxyl radical. Hydroxyl radical, peroxynitrite and peroxynitrite-derived products (hydroxyl radical, carbonate radical and nitrogen dioxide) all have the potential to react with and damage most cellular targets including lipids, proteins and DNA [110,111].

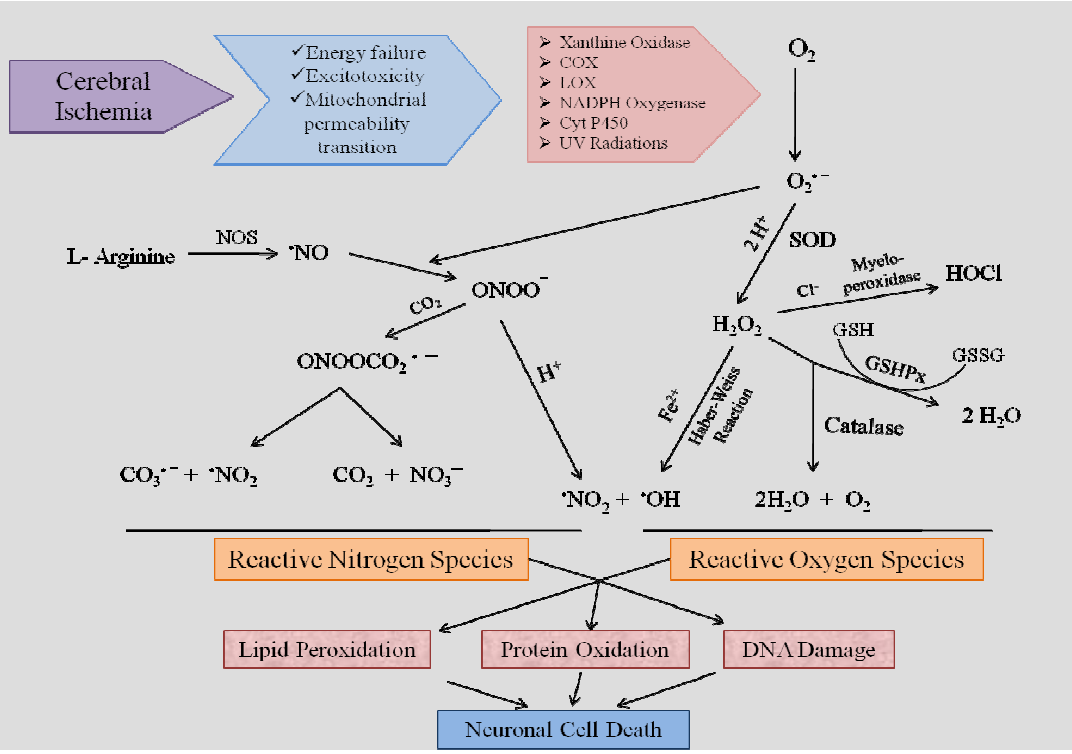

Figure 5.
Generation of Free Radicals in Cerebral Ischemic Stroke.

Cerebral Ischemia and Neuroinflammation

Few hours after the onset of ischemia, tissue injury begins with an inflammatory reaction, which is a common response of the cerebral parenchyma to various forms of insult [112,113]. This requires the infiltration of leukocytes, both polymorphonuclear (PMN) leukocytes and monocytes/macrophages (but not lymphocytes), which are the cellular mediators of subsequent microvessel obstruction, edema formation, cellular necrosis, and tissue infarction [114,115].

This inflammatory injury is induced by certain molecules includes cell adhesion molecules (selectins, integrins, and immunoglobulins), cytokines (IL-1, IL-6, TNF-α, and TGF-β) [116,117], Chemokines (CINC, MCP-1), inducible neuronal nitric oxide synthase (iNOS) produced by endothelial cells, activated astrocytes [118,119], microglial cells and leukocytes (granulocytes, monocyte/macrophages, and lymphocytes) and these all contribute to irreversible damage [120,121].

Cell adhesion molecules mediate cell to cell interaction for leukocyte migration. The recruitment of neutrophils to ischemic brain begins with neutrophil rolling on activated endothelial blood vessel walls, mediated by selectins, followed by neutrophil activation and adherence, mediated by integrins and immunoglobins [122,123,]. When adhered to cerebral blood vessel walls, neutrophils transmigrate into the cerebral parenchyma, a process facilitated by blood brain barrier (BBB) disruption. The recruitment of neutrophils can obstruct the microcirculation and prevent complete restoration of cerebral blood flow after reperfusion [124]. This blockage may cause further tissue damage after ischemia. Once neutrophils penetrate into ischemic brain they cause tissue damage by releasing oxygen free radicals and proteolytic enzymes. Further, Selectins are glycoproteins and comprised by P-, E-, and L-selectin. Members of the immunoglobulin supergene family, composed of Intracellular adhesion molecule (ICAM-1 and ICAM-2), vascular cell adhesion molecule–1 (VCAM-1; CD106), platelet-endothelial cell adhesion molecule–1 (CD31), mucosal addressin cell adhesion molecule–1 (CD146) and are expressed on activated endothelial cells [125,126].

Cytokines and Chemokines also contribute to stroke related brain injury. During ischemia, cytokines, such as interleukins (IL-1, IL-6), Tumor necrosis factor (TNF-α, TGF-β) and Chemokines such as Cytokine-induced neutrophil chemoattractant (CINC) and Monocyte chemoattractant protein–1 (MCP-1) are produced by a variety of activated cell types, including endothelial cells, microglia, neurons, platelets, leukocytes, and fibroblasts [127,128]. The possible deleterious effects of IL-1 include fever, arachidonic acid release; enhancement of NMDA mediated excitotoxicity and stimulation of nitric oxide synthesis. Both IL-1, TNF-α induces adhesion molecule expression in cerebral endothelial cells and promotes neutrophil accumulation and transmigration. In addition TNF-α stimulates acute-phase protein production, disrupts the blood-brain barrier and stimulates the induction of other inflammatory mediators. But TGF-β plays a neuroprotective role in the pathogenesis of stroke [129,130,131].

Ischemic Cell Death

Ischemia develops two zones around the site of blocked blood supply (thrombosis/embolism). Brain cells at the center of ischemic region where the cerebral circulation is completely arrested, this region called as core, irreversible cell damage occurs in several minutes[132]. In the periphery of this ischemic area, where collateral blood flow can buffer the full effects of the stroke, this region called as penumbra, the degree of ischemia and the timing of reperfusion determine the fate of individual cells[133]. The reduced blood flow falls to the level below the threshold for electrical failure and above the threshold for energy failure. Restoration of cerebral blood flow, even to a sub-optimal level, provides an opportunity for those brain cells to recover and regain functionality [134].

This infarction region has remarkably difference in the process of cellular injury and death [135]. There appear two major modes of

cell death that participate in ischemic cell death: necrosis and apoptosis. Necrosis as passive degeneration resulting in cellular dissolution when the internal homeostasis collapses. Apoptosis meaning active cellular suicide occurring during normal development and also being triggered by physiological stimuli [136]. **Aponecrosis** is another term given to apoptosis and necrosis in the brain infarction which seem like the two poles of a continuum of cellular death after ischemic stroke. While necrosis is more dominant in the core tissue, penumbral cells die by means of either mode, with apoptosis being more common for cells further away from the core [137].

Mechanisms of Necrotic Cell Death

Various morphological changes leading to necrosis includes swelling with blebbing of the cell surface, dilation of the endoplasmic reticulum, increased mitochondrial density, and flocculation of the nuclear chromatin which turns into irreversible cell swelling with mitochondrial dilation that results in rupture of nuclear membranes [138,139]. Before the loss of all basophilia, the marginated chromatin appears as small discrete masses. These changes, rupture of other organelle membranes and breakdown of plasma membrane makes cell boundaries indistinct followed by development of exudative inflammation in the adjoining viable tissue, and the debris is ingested and degraded by phagocytes [140,141].

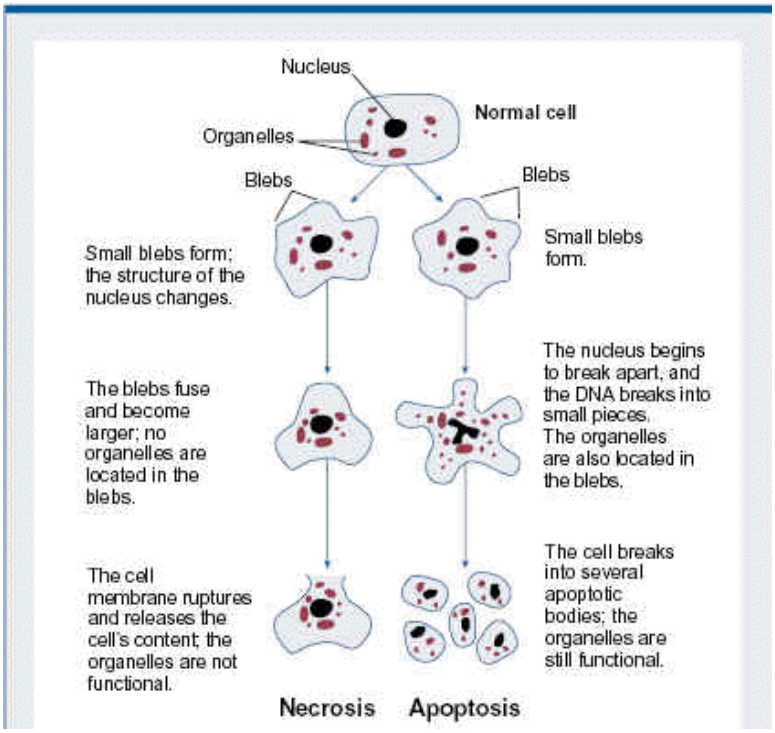

Figure 6.
Basic Characteristics of Ischemic Cell Death Modes: Necrosis and Apoptosis.

Mechanisms of Apoptotic Cell Death

Term apoptosis was coined by John Kerr and his group in 1972 which is a regulated and programmed phenomenon responsible for the maintenance of homeostasis of multicellular system that requires a various factors [142,143]. This process is involved in many biological events like development, differentiation, proliferation, immune system, and also removal of defective & harmful cells. Apoptosis means a program that triggered for death of a cell. Many of the key molecular events have determined in

cerebral ischemia like free radicals, Ca^{2+} overload mitochondria, excitotoxicity that initiates programmed cell death in many cells [144,145]. It is characterized by a series of well defined distinct morphological and biochemical changes that proceeds through progressive steps to avoid leakage of potentially harmful intracellular contents[146,147]. Mitochondria acts as reservoir for multiple apoptogenic proteins such as cytochrome c, SMAC/DIABLO, AIF, Endonuleases and procaspases -2,3,8,9. Cytochrome is released from mitochondria along with all other apoptogenic proteins which are involved in formation of apoptosome with apoptotic proteases-activating factor – 1 (APAF-1) and procaspase – 9. This activates Caspases – 9 that further cleaves and activates downstream Caspases such as caspase – 3,6,7. These Caspases degrades their substrates like endonucleases, lamin, spectrin, huntingtin, gelsolin, PARP, etc [148,149].

Also, there is activation of Death receptors (DRs) of TNF receptor family presents on cell surface [150]. These have specific death domains (DD) like TNF-receptor associated death domain (TRADD) or Fas-associated death domain (FADD) to which ligand binding promotes death inducing signaling complex (DISC) [151]. This also involves the activation of Bid, Bax. Another caspase independent and self destructive mechanism of cell death involves a novel apoptotic effector protein called AIF that resides in inner membrane space of mitochondria. This is responsible for chromatin condensation and large scale DNA fragmentation [152,153].

Cysteine **A**spartate **S**pecific **P**roteins**ases** also known as Caspases, the central molecules involved in initiation and execution of apoptosis [154]. The Caspase family is broadly divided into two categories: CED subfamily are activated during apoptosis constitute Caspases – 2,3,6,7,8,10 and ICE/Caspases –1 subfamily undergo activation during inflammatory responses constitute Caspases –1,4,5,11,12. Moreover, apoptotic Caspases can be further divided into initiating Caspases (-2,8,9,10,12, responsible for initiating the apoptosis) and effector Caspases (-3,6,7, actually involved in dismantling the cell) [155,156].

Endogenous Inhibitors of Apoptosis: Along with activation of proapoptotic proteins, there is also activation of inhibitors of apoptotic proteins (IAPs) [157,158]. Both balance and maintain the homeostasis of cell death. IAPs constitute a family of death suppressing proteins like c-IAP1, C-IAP2, X-linked IAP (XIAP) and surviving. Besides all these, certain heat shock proteins (HSPs) also interact with APaf 1 and preventing the constitution of apoptosome and subsequent caspase-9 activation [159,160].

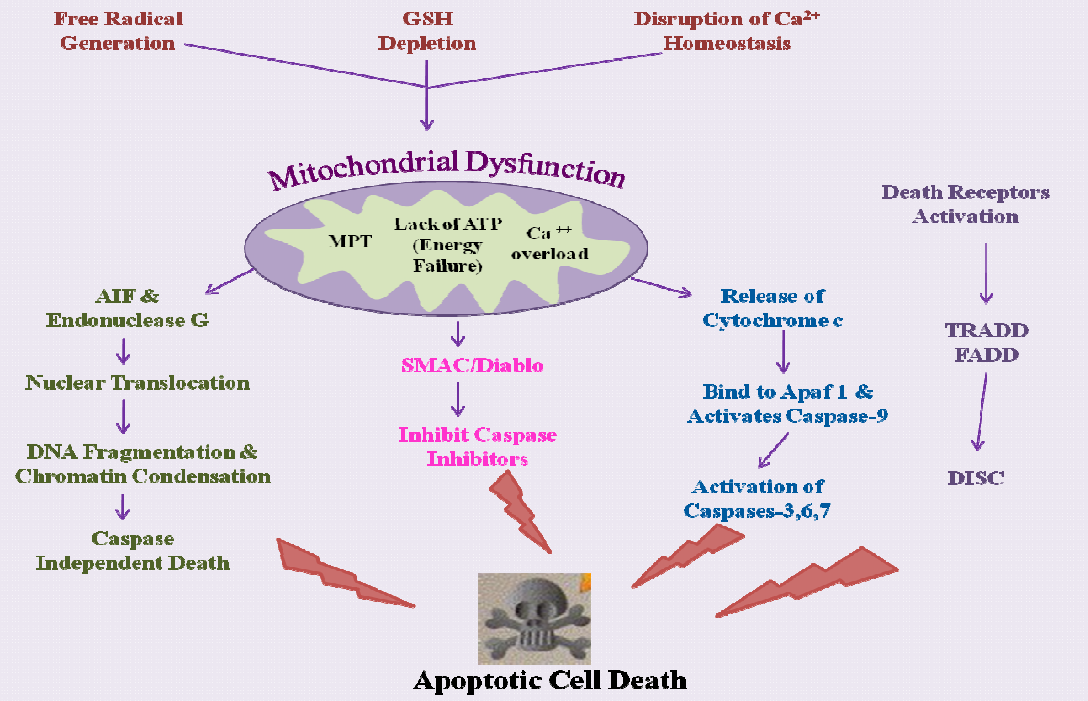

Figure 7.
Schematic Presentation of Apoptotic Cell Death.

NECROPTOSIS

Necroptosis is recently described by Degterev et al. (2005) as a series of pathway involving substantially non-apoptotic components that are involved in neuronal cell death which is caused by ischemic injury [161,162]. These involve death receptor mediated pathways of apoptosis that are followed by activation of Caspase-8. Further, in addition to initiation by death receptors they can also precede thro; RIP (Receptor interacting proteins) which differentiates this from death receptor pathway of apoptosis. RIP is a serine/threonine kinase and identified as a Fas-interacting protein, known to contain 3-domains named as N-terminal domain, C-terminal domain & an intermediate domain [163,164]. These domains bind to a TRADD, a TNF receptor I (TNFRI) associated cytoplasmic adapter protein which induces apoptosis indicating that RIP is a component of the TNFRI signaling complex. Three isoforms of RIP family have been identified, RIP2 (also known as CARDIAK/RICK), RIP3 and RIP4 (also known as DIK/PKK) [165]. An inhibitor of necroptosis, Necrostatin-1 (Nec-1) reported by Degterev et al. (2005) is shown to have good results in reducing the infarct volume and improving neurologic score resulting from MCAo mice. Therefore, necroptosis offers a new possibility to provide a new target of neuroprotection [166,167].

CHANGES IN NEUROTRANSMITTERS AND NEUROACTIVE SUBSTANCES

As stated by Durukan (2007), after postmortem (by immunohistochemical staining techniques) and in vivo (by microdialysis) evaluation of neurochemical changes in stroke induced animals that aspartate, glutamate, inosine, hypoxanthine, adenosine and γ-amino butyrate increases in the acute ischemic period [168,169] and glycine seems to increase with prolonged ischemia. Also there are some neuroactive substances like tyrosine hydroxylase, neuropeptide Y which increase in peri-infarct

region [170,171], but some substances like neuropeptide Y, leuenkephalin, neurotensin, and dynorphin get increased in nuclei of amygdale which are not infarcted. Similar results have been achieved with microdialysis method applied to patients with large MCA infarction [172,173,].

In most of the cases, the ischemic injury is reported to induce mild to severe permanent deficits[174,175]. Inspite of the increased level of these molecules, such level is further insufficient to produce its protective effects and to maintain normal physiology of brain [176,177]. There are other neurotransmitters (like dopamine, acetylcholine etc.) which gets decreased so as to maintain and regulate the normal behavior like coagulation, motor co-ordinations, active response to any stimuli etc. Among these neurotransmitters, acetylcholine has greatest amount in brain neuronal system and is also widely available[178,179]. Both, dopaminergic as well as acetylcholinergic neurotransmission critically modulates synaptic transmission and plasticity. In particular, acetylcholine acting at nicotinic and muscarinic receptors deeply influences the induction of LTP (Long Term Potentiation) and LTD (long term depression) in striatum, hippocampus and notably in several areas of brain implied in reward mechanisms. A wide range of attentional processes are mediated by forebrain cholinergic system[180,181,182]. Further, Dopamine critically regulates neuronal transmission and plasticity at cortico-striatal synapses, whose activity is crucial during formation of habits and skills. LTP is dopamine dependent in prefrontal cortex, hippocampus, and amygdale. Dopamine also involves in coordinating the movements of whole body skeletal muscles because it receives the stimuli in first nuclei (nigrostriatum) of basal ganglia where it balances with acetylcholine and send stimuli further to other nucleus [183,184].

Histopathological Changes in Cerebral Ischemia

Ischemic changes in cell architecture begin so rapidly that the brain interstitial space almost completely disappears within the few seconds of the onset of cerebral ischemia. Loss of interstitial space is a consequence of cell swelling secondary to sodium influx and failure of membrane ionic regulation[185,186].

After 10 minutes: A significant number of cells show clumping of nuclear chromatin and a modest increase in electron lucency after 10 minutes of GCI [187].

After 30 minutes: Further changes after 30 minutes include increased cytoplasmic swelling, swelling and shape change of the mitochondria, and some loss of mitochondrial matrix density. Microtubules disappear [188] and there is detachment of the ribosomes from the cisternae of the endoplasmic reticulum. There is also disassociation of the polyribosomes, and single ribosomes lose their compact structure with associated failure of protein synthesis [189].

After 60 minutes: One hour after GCI, the above changes have become more pronounced with more conspicuous swelling of the ER cisternae. The mitochondria begin to show slight inner matrix swelling and occasional flocculent densities [190].

After 120 minutes: Within 2-4 hours of GCI, the changes discussed above are more pronounced and a larger number of mitochondria exhibit the presence of flocculent densities evidencing calcium overload which is currently considered irreversible[191]. Published electron micrographs reveal intact lysosomes and seem to confirm other studies which indicate that lysosomal rupture and subsequent catastrophic autolysis is not a feature of early (1 - 4 hours) ischemic injury [192].

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