

Review Article



A Review on Pathogenesis of Cerebral Ischemia

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ABSTRACT

Current knowledge regarding the pathophysiology of cerebral ischemia and brain trauma indicates that similar mechanisms contribute to loss of cellular integrity and tissue destruction. Mechanisms of cell damage include excitotoxicity, oxidative stress, free radical production, apoptosis and inflammation. Genetic and gender factors have also been shown to be important mediators of patho mechanisms present in both injury settings. However, the fact that these injuries arise from different types of primary insults leads to diverse cellular vulnerability patterns as well as a spectrum of injury processes. Severe cerebral ischemic insults lead to metabolic stress, ionic perturbations, and a complex cascade of biochemical and molecular events ultimately causing neuronal death. Similarities in the pathogenesis of these cerebral injuries may indicate that therapeutic strategies protective following ischemia may also be beneficial after trauma. This review summarizes and contrasts injury mechanisms which leads to ischemia and trauma.

Keywords: Ischemic cascade, Excitotoxicity, Excitatory amino-acid transporters, Mitochondria.

INTRODUCTION

ISCHEMIC CASCADE

A cascade is a series of events in which one event triggers the next, in a linear fashion. Thus "ischemic cascade" is actually a misnomer, since in it events are not always linear, in some cases they are circular, and sometimes one event can cause or be caused by multiple events². In addition, cells receiving different amounts of blood may go through different chemical processes. Despite these facts, the ischemic cascade can be generally characterized as follows:³

1. Lack of oxygen causes the neuron's normal process for making ATP for energy to fail.
2. The cell switches to anaerobic metabolism producing lactic acid.
3. ATP-reliant ion transport pumps fail, causing the cell to become depolarized, allowing, ions including calcium (Ca^{++}), to flow into the cell.
4. The ion pumps can no longer transport calcium out of the cell, and intracellular calcium levels get too high.
5. The presence of calcium triggers the release of the excitatory amino acid neurotransmitter Glutamate.
6. Glutamate stimulates AMPA receptors and Ca^{++} -permeable NMDA receptors, which open to allow more calcium into cells.
7. Excess calcium entry overexcites cells and causes the generation of harmful chemicals like free radicals, reactive oxygen species and calcium-dependent enzymes such as calpain, endonucleases, ATPase, and phospholipases, in a process called Excitotoxicity^{4,5}. Calcium can also cause the release of more glutamate.

8. As the cell's membrane is broken down by phospholipases, it becomes more permeable, and more ions and harmful chemicals flow into the cell.
9. Mitochondria break down, releasing toxins and apoptic factors into the cell.
10. The caspase - dependent apoptosis cascade is initiated, causing cells to "commit suicide."
11. If the cell dies through necrosis, it releases glutamate and toxic chemicals into the environment around it. Toxins poison nearby neurons, and glutamate can over excite them.
12. If and when the brain is reperfused, a number of factors lead to reperfusion injury.
13. An inflammatory response is mounted, and phagocytic cells engulf damaged but still viable tissue.
14. Harmful chemicals damage the blood brain barrier.

Cerebral edema (swelling of the brain) occurs due to leakage of large molecules like albumins from blood vessels through the damaged blood brain barrier. These large molecules pull water into the brain tissue after them by osmosis. This "vasogenic edema" causes compression of and damage to brain tissue.

1. HYPOXIA

Cerebral hypoxia is a form of hypoxia (reduced supply of oxygen) specifically involving the brain when the brain is completely deprived of oxygen. There are four categories of cerebral hypoxia; in order of severity they are: 1. Diffuse cerebral hypoxia (DCH): A mild to moderate impairment of brain function due to low oxygen levels in the blood.



2. Focal cerebral ischemia: Is a stroke occurring in a localized area that can either be acute (sudden onset) and/ or transient (of short duration). This may be due to aneurysm, thrombus, embolus⁶.

3. Massive cerebral infarction: Is a "stroke", caused by complete oxygen deprivation due to an interference in cerebral blood flow which affects multiple areas of the brain.

4. Global cerebral ischemia: A complete stoppage of blood flow to the brain. Prolonged hypoxia induces neuronal cell death via apoptosis, resulting in a hypoxic brain injury⁷⁻⁹.

Cerebral hypoxia can also be classified by the cause of the reduced brain oxygen:

- Hypoxic hypoxia: Limited oxygen in the environment causes reduced brain function.
- Hypemic hypoxia: Reduced brain function is caused by inadequate oxygen in the blood despite adequate environmental oxygen.
- Ischemic hypoxia: Reduced brain oxygen is caused by inadequate blood flow to the brain.
- Histotoxic hypoxia: Oxygen is present in brain tissue but cannot be metabolized by the brain tissue.

Causes: Severe asthma and various sorts of anemia can cause some degree of diffuse cerebral hypoxia. Other causes include work in nitrogen rich environments, ascent from deep water dive, flying at high altitudes in an unpressurized cabin, and intense exercise at high altitudes prior to acclimatization, choking, drowning, strangulation, smoke inhalation, drug over doses, crushing of the trachea, status asthmaticus, and shock. It is also recreationally self-induced in the fainting game and in erotic asphyxiation. Transient Ischemic Attack (TIA): TIA is defined as a transient episode of neurologic dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction. The symptoms of a TIA can resolve within a few minutes unlike a stroke. TIAs and strokes present with the same symptoms such as contra lateral paralysis (opposite side of body from affected brain hemisphere), or sudden weakness or numbness. A TIA may cause sudden dimming or loss of vision, asyphasia slurred speech and mental confusion. The symptoms of a TIA typically resolve within 24 hours unlike a stroke. Brain injury may still occur in a TIA lasting only a few minutes. Having a TIA is a risk factor for eventually having a stroke^{10,11}. Silent stroke is a stroke which does not have any outward symptoms, and the patient is typically unaware they have suffered a stroke. Despite not causing identifiable symptoms a silent stroke still causes damage to the brain, and places the patient at increased risk for a major stroke in the future. Silent strokes typically cause lesions which are detected via the use of neuro imaging such as fMRI^{12,13}. The risk of silent stroke increases with age but may also affect younger adults. Women appear to be at increased risk for silent

stroke, with hypertension and current cigarette smoking being predisposing factors^{14,15}. The two main mechanisms are hypoxic and anoxia, Brain injury as a result of oxygen deprivation either due to hypoxic or anoxic mechanisms are generally termed hypoxic/anoxic injuries (HAI).

2. ACIDOSIS

Acidosis is said to occur when arterial pH falls. In mammals, the normal pH of arterial blood lies between 7.35 and 7.50 depending on the species. Changes in the pH of arterial blood (and therefore the extracellular fluid) outside this range result in irreversible cell damage^{16,17}. Although glucose is usually assumed to be the main energy source for living tissues, there are some indications that it is lactate, and not, glucose that is preferentially metabolized by neurons in the brain of several mammal species^{18,19}. According to the lactate-shuttling hypothesis, glial cells are responsible for transforming glucose into lactate, and for providing lactate to the neurons^{20,21}. Because of this local metabolic activity of glial cells, the extra cellular fluid immediately surrounding neurons strongly differs in composition from the blood cerebro spinal fluid, being much richer with lactate, as it was found in studies¹⁸. The role of lactate is being utilized by the brain even more preferentially over glucose¹⁸. It was also hypothesized that lactate may exert a strong action over GABAergic networks in the developing brain, making them more inhibitory²², acting either through better support of metabolites¹⁸ or alterations in base intracellular pH levels^{23,24} or both²⁵. The energy metabolism features in brain slices of mice and showed that beta-hydroxybutyrate, lactate and pyruvate acted as oxidative energy substrates causing an increase in the NAD(P)H oxidation phase, that glucose was insufficient as an energy carrier during intense synaptic activity and finally, that lactate can be an efficient energy substrate capable of sustaining and enhancing brain aerobic energy metabolism in vitro²⁶. Also provides novel data on biphasic NAD(P)H fluorescence transients, an important physiological response to neural activation that has been reproduced in many studies and that is believed to originate predominately from activity-induced concentration changes to the cellular NADH pools²⁷.

3. REPERFUSION INJURY

It is the tissue damage caused when blood supply returns to the tissue after a period of ischemia or lack of oxygen. The absence of oxygen and nutrients from blood during the ischemic period creates a condition in which the restoration of circulation results in inflammation and oxidative damage through the induction of oxidative stress rather than restoration of normal function. The inflammatory response is partially responsible for the damage of reperfusion injury. White blood cells carried to the area by the newly returning blood, release a host of inflammatory factor such as interleukins as well as free radicals in response to tissue damage²⁸. The restored blood flow reintroduces oxygen within cells that damages

cellular proteins, DNA and the plasma membrane. Damage to the cell's membrane may in turn cause the release of more free radicals. Such reactive species may also act indirectly in redox signaling to turn on apoptosis white blood cells may also bind to the endothelium of small capillaries, obstructing them and leading to more ischemia²⁸.

4. XANTHINE OXIDASE

In prolonged ischemia (60 minutes or more), hypoxanthine is formed as breakdown product of ATP metabolism. The enzyme hypoxanthine dehydrogenase acts in reverse, that is as a xanthine oxidase as a result of the higher availability of oxygen. This oxidation results in molecular oxygen being converted into highly reactive superoxide and hydroxyl radicals. Xanthine oxidase also produces uric acid, which may act as both a pro oxidant and as a scavenger of reactive species such as peroxy nitrite. Excessive nitric oxide produced during reperfusion reacts with superoxide to produce the potent reactive species peroxy nitrile. Such radicals and reactive oxygen species attack cell membrane lipids, proteins, and glycosaminoglycans, causing further damage. They may also initiate specific biological processes by redox nitrile. Reperfusion can cause hyperkalaemia²⁹.

5. GLUTAMATE

Glutamate is the most abundant excitatory neurotransmitter in the vertebrate nervous system³⁰. At chemical synopsis glutamate is stored in vesicles nerve impulses trigger release of glutamate from the pre-synaptic cell. In the opposing post-synaptic cell, glutamate receptors, such as the NMDA receptors, bind glutamate and are activated. Because of its role in synaptic plasticity, glutamate is involved in cognitive functions like learning and memory in the brain³¹. The form of plasticity known as long term potentiation takes place at glutamatergic synapses in the hippocampus, neocortex, and other parts of the brain. Glutamate works not only as a point-to-point transmitter but also through spill-over synaptic crosstalk between synapses in which summation of glutamate released from a neighboring synapse creates extra synaptic signaling/volume transmission³². Glutamate transporters are³³ found in neuronal and glial membranes. They rapidly remove glutamate from the extracellular space. In brain injury or disease, they can work in reverse, and excess glutamate can accumulate outside cells. This process causes calcium ions to enter cells via NMDA receptors channels, leading to neuronal damage and eventual cell death, and is called excitotoxicity. The mechanisms of cell death includes. Damage to mitochondria from excessively high intracellular calcium³⁴. Glu/Ca²⁺-mediated promotion of transcription factors for pro-apoptotic genes, or down regulation of transcription factors for anti-apoptotic genes. Excitotoxicity due to excessive glutamate release and impaired uptake occurs as part of the ischemic cascade and is associated with stroke and diseases like amyotrophic lateral sclerosis, lathyrism, autism, some

forms of mental retardation, and Alzheimer's disease³⁵. In contrast, decreased glutamate release is observed under conditions of classical phenyl ketonuria leading to developmental disruption of glutamate receptors expression.

6. EXCITATORY AMINO-ACID TRANSPORTERS (EAATs)

It is also known as glutamate transporters, belong to the family of transporters Glutamate is the principal excitatory neurotransmitter in the vertebrate brain. EAATs serve to terminate the excitatory signal by removal (uptake) of glutamate from the neuronal synapse into neuralgia and neurons. The EAATs are membrane-bound secondary transporters that superficially resemble ion channels³⁶. These transporters play the important role of regulating concentrations of glutamate in the extra cellular space by transporting it along with other ions across cellular membranes³⁷. After glutamate is released as the result of an action potential, glutamate transporters quickly remove it from the extracellular space to keep its levels low, thereby terminating the synaptic transmission^{36,38}. Without the activity of glutamate transporters, glutamate would build up and kill cells in a process called excitotoxicity, in which excessive amounts of glutamate acts as a toxin to neurons by triggering a number of biochemical cascades. The activity of glutamate transporters also allows glutamate to be recycled for repeated release³⁹. Over activity of glutamate transporters may result in inadequate synaptic glutamate and may be involved in schizophrenia and other mental illnesses⁴⁰. During injury processes such as ischemia and traumatic brain injury, the action of glutamate transporters may fail, leading to toxic buildup of glutamate. In fact, their activity may also actually be reversed due to inadequate amounts of adenosine triphosphate to power ATPase pumps, resulting in the loss of the electro chemical ion gradient. Since the direction of glutamate transport depends on the ion gradient, these transporters release glutamate instead of removing it, which results in neurotoxicity due to over activation of glutamate receptors⁴¹. Loss of the Na⁺-dependent glutamate transporter EAAT2 is suspected to be associated with neuro degenerative diseases such as Alzheimer's disease, Huntingtons disease, and ALS-parkinsonism dementia complex⁴². Also degeneration of motor neurons in the disease amyotrophic lateral sclerosis has been linked to loss of EAAT2 from patients' brains and spinal cords⁴².

7. MITOCHONDRIA

Mitochondria produce more than 90% of our cellular energy by ox-phos^{43,44}. Energy production is the result of two closely coordinated metabolic processes — the tricarboxylic acid (TCA) cycle, also known as the Krebs or citric acid cycle, and the electron transport chain (ETC). The TCA cycle converts carbohydrates and fats into some ATP, but its major job is producing the coenzymes NADH and FADH so that they, too are entered into the ETC. NADH and FADH carry electrons to the ETC, which is



embedded in the inner mitochondrial membrane and consists of series of five enzyme complexes, designated I–V. Electrons donated from NADH and FADH flow through the ETC complexes, passing down an electrochemical gradient to be delivered to diatomic oxygen (O₂) via a chain of respiratory proton (H⁺) pumps⁴⁵. Complexes I–IV involve ubiquinone (Coenzyme Q10, which is embedded in the inner mitochondrial membrane and consists of a series of five enzyme complexes, designated I–V. Complexes I–IV involve ubiquinone. Complex II is succinate dehydrogenase (SDH), complex III is the bcl complex, complex IV is cytochrome c oxidase (COX), and complex V is ATP synthase⁴⁴. Complexes I–IV contain flavins, which contain riboflavin, iron-sulfur clusters, copper centers, or iron containing heme moieties. Ubiquinone shuttles electrons from complexes I and II to complex III. Cytochrome c, an iron-containing heme protein with a binuclear center of a copper ion⁴⁶, transfers electrons from complex III to IV. During this process, protons are pumped through the inner mitochondrial membrane the inter membrane space to establish a proton motive force, which is used by complex V to phosphorylate adenosine diphosphate (ADP) by ATP synthase, thereby creating ATP. Proper functioning of the TCA cycle and ETC requires all the nutrients involved in the production of enzymes and all the cofactors needed to activate the enzymes.

Mechanisms includes damage to mitochondria is caused primarily by reactive oxygen species (ROS) generated by the mitochondria themselves^{47,48}. It is currently believed that the majority of ROS are generated by complexes I and III⁴⁹ likely due to the release of electrons by NADH and FADH into the ETC. Mitochondria consume approximately 85% of the oxygen utilized by the cell during its production of ATP.⁵⁰ During normal oxphos, 0.4–4.0% of all oxygen consumed is converted in mitochondria to the superoxide (O₂⁻) radical⁵⁰⁻⁵². Superoxide is transformed to hydrogen peroxide (H₂O₂) by the detoxification enzymes manganese superoxide dismutase (MnSOD) or copper/zinc superoxide dismutase (Cu/Zn SOD)⁵³ and then to water by glutathione peroxidase (GPX) or peroxiredoxin III (PRX III)⁵⁴. However, when these enzymes cannot convert ROS such as the superoxide radical to H₂O fast enough, oxidative damage occurs and accumulates in the mitochondria^{55,56}. Glutathione in GPX is one of the body's major antioxidants. Additionally, nitric oxide (NO) is produced within the mitochondria by mitochondrial nitric oxide synthase (mtNOS)⁵⁷ and also freely diffuses into the mitochondria from the cytosol⁵⁸. NO reacts with O₂⁻ to produce another radical, peroxynitrite (ONOO⁻)⁵⁸. Complex I is especially susceptible to nitric oxide (NO) damage, and animals administered natural and synthetic complex I antagonists have undergone death of neurons⁵⁹⁻⁶¹. Complex I dysfunction has been associated with Leber hereditary optic neuropathy, Parkinson's disease, and other neurodegenerative conditions^{62,63}. Together, these two radicals as well as others can do great damage to mitochondria and other cellular within the mitochondria,

elements that are particularly vulnerable to free radicals include lipids, proteins, oxidative phosphorylation enzymes, and (mtDNA)^{64,65}. Direct damage to mitochondrial proteins decreases their affinity for substrates or coenzymes and, thereby, decreases their function⁶⁶. Compounding the problem, once a mitochondrion is damaged, mitochondrial function can be further compromised by increasing the cellular requirements for energy repair processes⁶⁷. Mitochondrial dysfunction can result in a feed forward process, whereby mitochondrial damage causes additional damage.

8. INFLAMMATORY MEDIATORS

Inflammatory mediators such as tumor necrosis factor alpha (TNF-α) have been associated in vitro with mitochondrial TNF-α results in mitochondrial dysfunction by reducing complex III dysfunction and increased ROS generation⁴⁷. Medical research has found that iron-deficiency anemia is a major factor. Low iron status decreases mitochondrial activity by causing a loss of complex IV and increasing oxidative stress⁶⁸. Toxic metals, especially mercury, generate many of their deleterious effects through the formation of free radicals, resulting in DNA damage, lipid peroxidation, depletion of protein sulfhydryls (e.g. glutathione) These reactive radicals include a wide-range of chemical species, including oxygen-, carbon-, and sulfur radicals originating from the superoxide radical, hydrogen peroxide, lipid peroxides, and also from chelates of amino acids, peptides, and proteins complexed with the toxic met One major mechanism for metals toxicity appears to be direct and indirect damage to mitochondria via depletion of glutathione, an endogenous thiol-containing (SH-) antioxidant which results in excessive free radical generation and mitochondrial damage⁶⁹.

CONCLUSION

Ischemic cascade which follows the chain reaction in which it mainly starts with the hypoxic conditions it switches to anaerobic respiration followed by acidosis condition which activates glutamate AMPA, NMDA. This leads to generation of free radicals causes excitotoxicity, necrosis. Xanthine oxidase which produces super oxide, hydroxyl radicals and uric acid which triggers to peroxynitrite. These radicals react with oxygen species attack the membrane lipids, proteins causes further damage. Overall the formation of free radicals mainly causes the severe ischemia, so the herbal plants which are having anti oxidant activity shows much effectively to treat ischemia.

REFERENCES

1. Helen M Bramlett and Dalton Dietrich W, Journal of cerebral blood flow, 24, 2004, 133-150.
2. Hinkle JL, Bowman L, Neuro protection for ischemic stroke. J Neurosci Nurs 35 (2), 2003, 114–8.
3. Ischemic cascade. http://en.wikipedia.org/wiki/Ischemic_cascade (accessed 25th feb 2013).



4. Jill Conway. 2000. "Disease at the cellular level lecture hand out " and "Inflammation and repair lecture Hand out "University of Illinois College of Medicine. Retrieved on January 9, 2007.
5. eMedicine - Acute Stroke Management : Article by Edward C Jauch.
6. Pressman BD, Tourje EJ, Thompson JR, AJR Am J Roentgenol. An early CT sign of ischemic infarction: increased density in a cerebral artery. Sep:149(3), 1987, 583-6.
7. Malhotra R. Hypoxia induces apoptosis via two independent pathways in Jurkat cells: differential regulation by glucose. Am J Physiol Cell Physiol. Nov: 281(5), 2001,1596-603.
8. Mattiesen WR.Increased neurogenesis after hypoxic-ischemic encephalopathy in humans is age related. Acta Neuropathol. May:117(5), 2009, 525-34.
9. "HYPOXIA". The Gale Encyclopedia of Neurological Disorders. The Gale Group, Inc. 2005. Retrieved on 2007-04-13.
10. Ferro JM et al. Diagnosis of transient ischemic attack by the non neurologist. A validation study. Dec:27(12), 1996, 2225-9.
11. Easton JD. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease. The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. Stroke. Jun: 40(6), 2009, 2276-93.
12. Herdersche D. Silent stroke in patients with transient ischemic attack or minor ischemic stroke. The Dutch TIA Trial Study Group. Stroke. Sep: 23(9), 1992,1220-4.
13. Leary MC, Saver JL. Annual incidence of first silent stroke in the United States: a preliminary estimate. Cerebrovasc Dis.16(3), 2003,280-5.
14. Vermeer SE, Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. Stroke. Jan:33(1), 2002, 21-5.
15. Herdersche D, Silent stroke in patients with transient ischemic attack or minor ischemic stroke. The Dutch TIA Trial Study Group. Stroke. Sep: 23(9), 1992,1220-4.
16. Bo K. SIESJOM, D.Pathophysiology and treatment of focal cerebral ischemia Part 11: Mechanisms of damage and treatment Bo K. J Neurosurg.:77, 1992, 337-354.
17. 17 .Needham, A Comparative and Environmental Physiology. Acidosis and Alkalosis. 2004
18. Zilberter Y, Zilberter T, Bregestovski P "Neuronal activity in vitro and the in vivo reality: the role of energy homeostasis". Trends Pharmacol. Sci. 31 (9), 2010,394–401.
19. 19 .Wyss MT, Jolivet R, Buck A, Magistretti PJ, Weber B "In vivo evidence for lactate as a neuronal energy source". J. Neurosci. 31(20),2011, 7477–85.
20. Gladden LB. "Lactate metabolism: a new paradigm for the third millennium". J. Physiol. 558, 2004, 5–30.
21. Pellerin L, Bouzier-Sore AK, Aubert A. "Activity-dependent regulation of energy metabolism by astrocytes: an update". Glia 55 (12), 2007, 1251–62.
22. Holmgren CD, Mukhtarov M, Malkov AE, Popova IY, Bregestovski P, Zilberter Y. "Energy substrate availability as a determinant of neuronal resting potential, GABA signaling and spontaneous network activity in the neonatal cortex in vitro". J. Neurochem. 112 (4), 2010,900–12.
23. Tyzio R, Allene C, Nardou R.. "Depolarizing actions of GABA in immature neurons depend neither on ketone bodies nor on pyruvate". J. Neurosci. 31 (1), 2011, 34–45.
24. Ruusuvuori E, Kirilkin I, Pandya N, Kaila K. "Spontaneous network events driven by depolarizing GABA action in neonatal hippocampal slices are not attributable to deficient mitochondrial energy metabolism". J. Neurosci. 30 (46),2010, 15638–42.
25. Khakhalin AS. "Questioning the depolarizing effects of GABA during early brain development". J Neurophysiol. 106 (3), 2011, 1065–7.
26. Zilberter, Yuri; Bregestovski, Piotr; Mukhtarov, Marat; Ivanov, Anton. "Lactate Effectively Covers Energy Demands during Neuronal Network Activity in Neonatal Hippocampal Slices". Frontiers in Neuroenergetics. 3: (2), 2011
27. Kasischke, Karl "Lactate Fuels the Neonatal Brain". Frontiers in Neuroenergetics.2011.
28. Clark, Wayne M.. "Reperfusion Injury in Stroke". eMedicine. WebMD. Retrieved 2006-08-09.
29. John L. Atlee . Complications in anesthesia. Elsevier Health Sciences.July: 55, 2010.
30. Meldrum, B. S. "Glutamate as a neurotransmitter in the brain: Review of physiology and pathology". The Journal of nutrition 130 (4),2000, 1007–1015.
31. Mc Entee, W. J Crook, T. H. "Glutamate: Its role in learning, memory, and the aging brain". Psychopharmacology. 111 (4),1993, 391–401.
32. Okubo Y, Sekiya H, Namiki S, Sakamoto H, Iinuma S, Yamasaki M, Watanabe M, Hirose K. "Imaging extra synaptic glutamate dynamics in the brain". Proceedings of the National Academy of Sciences. 107 (14), 2010, 6526.
33. Shigeri Y, Seal R. P, Shimamoto, K. "Molecular pharmacology of glutamate transporters, EAATs and VGLUTs". Brain Research Reviews. 45 (3),2004, 250–265.
34. Manev H, Favaron M, Guidotti A, Costa E. "Delayed increase of Ca²⁺ influx elicited by glutamate: Role in neuronal death". Molecular Pharmacology 36 (1), 1989, 106–112.
35. Hynd M, Scott H.L, Dodd P. R. "Glutamate-mediated excitotoxicity and neuro degeneration in Alzheimer's disease". Neurochemistry International .45 (5), 2004,583–595.
36. Ganel R, Rothstein JD." Glutamate transporter dysfunction and neuronal death". In Monyer, Hannah; Gabriel A. Adelman; Jonas, Peter. Ionotropic glutamate receptors in the CNS. Berlin: Springer. 1999, 472–493. Zerangue N, Kavanaugh MP . "Flux coupling in a neuronal glutamate transporter". Nature. 383 (6601),1996, 634–37.
37. Shigeri Y, Seal RP, Shimamoto K. "Molecular pharmacology of glutamate transporters, EAATs and VGLUTs". Brain Res. Brain Res. Rev. 45 (3), 2004,250–65.
38. Zou JY, Crews FT "TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition". Brain Res. 1034 (1-2),2005, 11–24.
39. Ganel R, Rothstein JD. "Glutamate transporter dysfunction and neuronal death". In Monyer, Hannah; Gabriel A. Adelman; Jonas, Peter. Ionotropic glutamate receptors in the CNS. Berlin: Springer. 15,1999,472–493.
40. Kim AH, Kerchner GA, Choi DW ." Blocking Excitotoxicity". In Marcoux, Frank W. CNS neuroprotection. Berlin: Springer. 1,2002, 3–36.
41. Yi JH, Hazell AS ."Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury". Neurochem. Int. 48 (5), 2006, 394–403.
42. Steve R. Piezenik, John Neustadt. Mitochondrial dysfunction and molecular pathways of disease. Experimental and Molecular Pathology.83, 2007,84–92.

43. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* jul, 59 (3),1979, 527–605
44. Brookes P.S, Yoon Y, Robotham J, Anders M.W, Sheu S.S. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell. Physiol.* 287 (4), 2004,817–833.
45. Hunter D.J, Oganessian V.S, Salerno J.C, Butler C.S, Ingledew W.J, Thomson A.J. Angular dependences of perpendicular and parallel mode electron paramagnetic resonance of oxidized beef heart cytochrome c oxidase. *Biophys. J.* jan: 78 (1), 2000,439–450 .
46. Wei Y.H, Lu C.Y, Lee H.C, Pang C.Y, Ma Y.S. 1998. Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function. *Ann. N. Y. Acad. Sci.* 854, 1998,155–170 .
47. Duchen M.R. Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Mol. Aspects Med.* 25 (4),2004, 365–451 .
48. Harper M.E, Bevilacqua L,Hagopian K, Weindrich R, Ramsey J.J. Ageing, oxidative stress, and mitochondrial uncoupling. *Acta. Physiol. Scand.* 182 (4),2004, 321–331 .
49. Shigenaga M, Hagen T, Ames B. Oxidative damage and mitochondrial decay in aging. 91 (23),1994, 10771–10778.
50. Evans J.L, Goldfine I.D, Maddux B.A, Grodsky G.M. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of Type 2 diabetes. *Endocr. Rev.* 23 (5), 2002,599–622.
51. Carreras M.C, Franco M.C, Peralta J.G, Poderoso J.J. Nitric oxide, complex I, and the modulation of mitochondrial reactive species in biology and disease. *Mol. Aspects Med.* 25 (1–2), 2004,125–139.
52. Wallace D.C. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annual Review of Genetics.* 39 (1), 2005,359–407.
53. Green K, Brand M.D, Murphy M.P. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes* 53, 2004, 110–118.
54. James A.M, Murphy M.P. How mitochondrial damage affects cell function. *J. Biomed. Sci.* 9 (6), 2002,475–487.
55. Sies H. Strategies of antioxidant defense. *Eur. J. Biochem.* 215 (2),1993, 213–219.
56. Carreras M.C, Franco M.C, Peralta J.G, Poderoso J.J. Nitric oxide, complex I, and the modulation of mitochondrial reactive species in biology and disease. *Mol. Aspects Med.* 25 (1–2), 2004,125–139 .
57. Green K, Brand M.D, Murphy M.P. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes* 53 (1),2004,110–118.
58. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron.* 39 (6), 2003,889–909 .
59. Betarbet R, Sherer T.B, MacKenzie G, Garcia-Osuna M, Panov A.V, Greenamyre J.T. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3 (12),2000, 1301–1306 .
60. Qi X, Lewin A.S, Hauswirth W.W, Guy J. 2003. Suppression of complex I gene expression induces optic neuropathy.*Ann.Neurol.* 53 (2),2003, 198–205.
61. Schon E.A, Manfredi G. Neuronal degeneration and mitochondrial dysfunction. *J. Clin. Invest.* 111(3), 2003, 303–312.
62. Perier C, Tieu K, Guegan C. Complex I deficiency primes Baxdependent neuronal apoptosis through mitochondrial oxidative damage. *Proc. Natl. Acad. Sci. U. S. A.* 102 (52), 2005, 19126–1913.
63. Shigenaga M, Hagen T, Ames B. Oxidative damage and mitochondrial decay in aging. *PNAS.* 91 (23),1994,10771–10778.
64. Tanaka M, Kovalenko S.A, Gong J.S. Accumulation of deletions and point mutations in mitochondrial genome in degenerative diseases. *Ann. N. Y Acad. Sci.* 786,1996,102–111.
65. Liu J, Killilea D.W, Ames B.N. Age-associated mitochondrial oxidative decay: improvement of carnitine acetyltransferase substratebinding affinity and activity in brain by feeding old rats acetyl-L-carnitine and/or R-alpha-lipoic acid. *Proc. Natl. Acad. Sci. U. S. A.* 99 (4),2002, 1876–1881.
66. Aw T.Y, Jones D.P. Nutrient supply and mitochondrial function. *Annu. Rev. Nutr.* 9,1989, 229–251.
67. David W. Mc Candless, Ataman, Metabolic Encephalopathy. Houghton 2009. 504.
68. S.J. Jamesa, William Slikker IIIb, Stepan Melnyka, Elizabeth Newbw, Marta Pogribnab, Stefanie Jernigana. Thimerosal Neurotoxicity is associated with Glutathione Depletion: Protection with Glutathione Precursors. *NeuroToxicology.*2004.

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