This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

HIV and AIDS Dementia: a killer in disguise!

By

Irma Nydegger

The University of Iowa
Department of Chemistry

Iowa City, IA 52242-1294

For 77:222, Spring 2005
May 5, 2005

Abbreviations

AIDS:	acquired immunodeficiency syndrome
APC:	antigen presenting cells
EAA:	excitatory amino acid
HIV:	human immunodeficiency virus
IL-1β:	interleukin-1β
NMDA:	N-methyl-D-aspartate
NOS:	nitric oxide synthase
PAF:	platelet-activating factor
RNAse:	ribonuclease
SIVcpz:	simian immunodeficiency virus in chimpanzees
SIVgsn:	simian immunodeficiency virus in greater spot-nosed monkeys
SIVrcm:	simian immunodeficiency virus in red-capped monkeys
TNF-α:	tumor necrosis factor- α
GTP:	guanosine triphosphate
cGMP:	cyclic guanosine monophosphate

Table of Contents

Abstract:

AIDS (acquired immunodeficiency syndrome) dementia is a form of dementia that is observed in HIV (human immunodeficiency virus) patients during the late stages of AIDS. Treatment of AIDS with antiretroviral therapy has proven valuable for controlling the disease in the body, however these drugs cannot reach the brain and the infection can proceed unchecked. Like other forms of dementia, patients with this disease exhibit neuronal cell death even though the neurons are not directly infected. The HIV crosses the blood-brain barrier through infected T-helper cells and attacks the microglia. The infected microglia will release neurotoxins that trigger a cascade of events ultimately leading to neuronal cell death. These neurotoxins include free radicals like $NO^{\bullet}$ and $O_2^{\bullet -}$, cytokines and the excitatory amino acid glutamate. Cytokines and glutamate will overexcite NMDA (N-methyl-D-aspartate) receptors leading to a sharp increase of intracellular Ca^{2+} levels. This increase triggers the activation of many enzymes that compromise the integrity of the neuronal cell. The free radicals will also initiate unwanted oxidations and will mediate cell death. The scope of this review is the AIDS dementia symptoms, mechanism of infection, and the proposed routes of neuronal cell death.

Introduction

AIDS dementia is a rapidly growing disease among HIV infected patients. It belongs to the class of diseases that are part of the dementia family. The long standing paradox of this disease was the fact that massive neuronal cell death was observed, but the microglia were actually infected by the HIV. So how does the infection spread to the neurons eventually leading to their death? The mechanism of infection has proven to be intricate and complicated, but understanding how the infection causes neuronal cell death may be the key to providing potential new treatments for this disease.

Dementia

Approximately two million people suffer from some form of severe dementia. Five to eight percent of people over the age of 65 live with some type of dementia and these numbers double every five years after 65. Dementia is a category of brain diseases that are characterized by a loss in cognitive function[*]. This loss is brought on by either trauma or disease. Dementia can be permanent or reversible depending on how the changes in the brain occur. Cognition is a very important process involving the way humans perceive, learn, think and communicate with other humans[*]. Dementia may impair one or all of these areas of cognition: verbal communication, memory, judgment, spatial orientation and reasoning. Dementia can also induce changes in personality and behavior and as such is a very painful and emotional disease. Some of the risks involved with the development of dementia besides age are head traumas, deficiencies in vitamins such as B_{12}, B_1 and folic acid, cardiovascular diseases, kidney, thyroid or liver diseases etc[*]. Genetic make up is also very important in the development of dementia. AIDS

[*] From http://www.neurologychannel.com/dementia/ accessed on 4/15/05

dementia is among the diseases that fall under the dementia category (other forms include Alzheimer's, Parkinson's, Lou Gehrig's disease etc)

AIDS dementia

Symptoms

In 1990 an estimated 20-30% of patients living with HIV showed symptoms of AIDS dementia [1]. This disorder is the most prevalent form of dementia among people that are 40 years of age or younger and can be a significant source of death from AIDS [1]. AIDS dementia is very similar in nature to other dementia disorders such as Alzheimer's disease. Approximately half of children and a third of adults that suffer from AIDS express symptoms of dementia and neurological disorders [2, 3]. These symptoms include those observed for other types of dementia, such as loss of cognitive function, loss of coordination and movement ability and loss of sensation [2, 3]. Neurological symptoms of the disorder consist of penetration of the brain by monocytoid cells, astrocytosis, myelin pallor, condensing of dendrites and eventually cell loss [3]. AIDS dementia is ultimately caused from the HIV infection of the brain. To understand the mechanism of infection it is important to first look at the HIV itself.

HIV: history and mechanism of infection

1.1 HIV Origin

A 2004 estimate placed the number of people that live with the HIV/AIDS at 39.4 million worldwide[§]. Also in 2004, 3.1 million people died of the disease and 4.9 million others were diagnosed with HIV[§]. There is little doubt that most of the world's population has at some point in time been exposed to the concept of HIV, its epidemic and consequences. But how did this virus originate?

Little is known about the HIV infection prior to 1970's. This could be because the spread of the infection was not accompanied by symptoms significant enough to be detected[§]. There are documented cases of the disease before the 1970's, but the spread that is observed today began in the mid to late 1970's and by 1980's HIV was spread to all five continents. The first cases of AIDS appeared in the US in 1981, but initially it was not clear what the source of the disease was. Today it is well established that AIDS is caused by the HIV virus.

The first person to be attributed with the discovery of HIV is a French scientist named Luc Montagnier in 1984[‡]. HIV is a retrovirus belonging to the lentivirus family. These viruses infect primarily primates. They are also known as SIV (simian or monkey immunodeficiency viruses) [§]. There are two types of HIV viruses, HIV-1 and HIV-2. In humans, these viruses are cross-species or zoonotic [4]. HIV-2 has been pinpointed to come from a primate species called *Cercocebus atys* or sooty mangabey. The origin of HIV-1, the more dangerous virus of the two, has remained in question [4]. HIV-1 type virus is believed to be a direct descendent of the simian immunodeficiency virus (SIVcpz) that was isolated from chimpanzees (*Pan troglodytes*) in west central Africa. SIVcpz is believed to descend from recombinations and cross-species transmissions of SIV that infects monkeys [5]. Phylogenic studies have shown that the SIVcpz virus itself has the origin in a cross between SIVrcm and SIVgsn that infect red-capped monkeys and greater spot-nosed monkeys respectively [5]. Little is known on how this virus crossed species and infected humans. Based on computer model studies, it is speculated that the cross over of the SIV to humans may have occurred as early as the 1930s (with a 20 year

[§] From http://www.avert.org/his81_86.htm accessed on 4/11/05
[‡] From http://www.biol.sc.edu/courses/bio102/hiv.html accessed on 4/13/05

margin of error) with the oldest reported case of AIDS death occurring in 1959 [6].

Understanding the origin and evolution of HIV-1 is important because it can provide insight on how to fight the disease. Knowledge of HIV-1 rapid mutations from the point of origin can present better treatment options and also help prevent future epidemics of these types of viruses. So how does HIV-1 infect humans and how is it expressed?

1.2 HIV Structure

As was mentioned above, HIV strains are retroviruses, which means that their genetic information is packed into their RNA. Figure 1 depicts the general structure of the HIV-1 strain[§].

The outer layer of the virus is comprised of a viral lipid bilayer envelope that the virus incorporates from the infected cells. This lipid bilayer contains glycoproteins gp120 and gp41. The latter non-covalently binds to gp120 and mediates the fusion of the viral membrane to the cellular membrane. The inside of the viral envelope contains proteins that surround the RNA and the reverse transcriptase[‡].

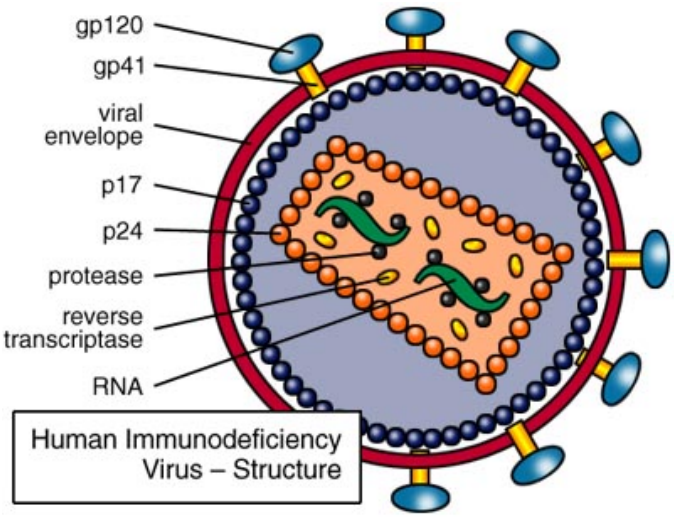

Figure 1. HIV structure.
Picture taken from http://www.avert.org/his81_86.htm (accessed on 4/11/05)

1.3 Target

There are three types of defense mechanisms in the human body. The first mechanism is external

and it is represented by the mucous membranes and the skin. The HIV does not penetrate against the outer defense mechanism. HIV is somewhat easy to control, unlike airborne viruses‡. The second defense mechanism is nonspecific and is internal. This mechanism involves phagocytes and natural killer cells that destroy microbes and endogenous infected cells, but they do not involve memory. The third type of defense mechanism is also internal. It is divided into two main branches: the humoral response and the cell-mediated response. Unlike the phagocytes and the natural killer cells, these immune system cells are very specific and involve memory. The humoral response is mediated by lymphocyte B cells and the cell-mediated response entails a more complex network of lymphocytes and among them the T-helper cells.

The HIV virus, once it gets incorporated into the body will attack the lymphocytes and specifically T-helper cells. A membrane bound protein called CD4 is expressed on the surface of T-helper cells. CD4 is also expressed in macrophages in lungs and brain‡. This protein recognizes the antigens that are bound to the APC (antigen presenting cells) or macrophages‡. Besides binding to macrophages, CD4 proteins also bind to the glycoprotein gp120 of the viral envelope of the HIV.

1.4 **Mechanism of infection**

The first step in the mechanism of infection of HIV is the binding of the glycoprotein gp120 of the viral envelope to the CD4 receptors of the T-helper cells of the immune system‡. Once the binding happens, the HIV gp41 glycoprotein facilitates the fusion of the two membranes: the virus and the host membranes. After the fusion is completed then the genetic material of HIV enclosed in the RNA gets released into the T-helper cell‡. The second step in this mechanism involves the viral RNA reverse transcriptase protein, which transcripts the RNA into the DNA of the host forming a

hybrid RNA-DNA molecule‡. The RNA is then degraded by the host's RNAse and a matching DNA strand is synthesized producing a double stranded DNA. In the third step, the newly produced double DNA strand travels to the host's nucleus‡. It then incorporates the host's genetic material and enters the latent or noninfectious stage. At this stage, the detection of the infection is almost nonexistent, because there are no antibodies produced yet. The viral genes are then transcribed into RNA‡. One part is mRNA that encodes the translation of viral proteins and the other part is viral RNA which encodes the genetic material of the virus. The last step involves the production of a new virus (viral RNA and proteins) and the viral envelope which is developed from the plasma membrane of the host‡.

Infected T-helper cells that release these newly formed viruses can either die or express the membrane glycoproteins of the HIV virus (gp120) ‡. The expression of the gp120 on the surface of the infected T-helper cells causes them to bind to CD4 receptors of uninfected T-cells and merge together. This process, called syncytium, creates a cytoplasm mass that has many nuclei but no internal cell boundaries and is fatal to T-helper cells‡. The newly secreted HIV can also attack uninfected T-helper cells and continue the cycle causing a severe depletion of these immune system cells‡. The question then becomes how does the HIV infection invade the brain and what is the mechanism of action? This question however warrants a quick overview of the central nervous system.

The Central Nervous System

The nervous system is comprised of two main types of cells: neurons and glia. There are approximately 100 billion neurons in the human brain and ten times as many glia cells [7]. Neurons take part in the function of the brain, such as sensing changes

in the environment, communicating these changes to neighboring neurons and control the body's responses to these external stimuli. Much less is known about glia cells. They are believed to be the support system for neurons and insulate and nourish them[7]. Future scientific work may change today's perspective on the function and importance of these cells. Neurons can be categorized into: unipolar, bipolar and multipolar. Most neurons are multipolar, meaning they possess multiple neurites (axons and dendrites) [7]. Figure 2. General scheme of a typical neuron.

Glia cells are divided into: astrocytes, oligodendrocytes. Astrocytes are star shaped cells that control and regulate the chemical content of the space around neurons [7]. Oligodendrocytes are smaller than astrocytes and their main function is the myelination of axons of the central nervous system. Other cells that are found in the nervous system are: schwann cells that form the myelin sheaths of the peripheral nervous system, and microglia, whose role is to remove waste products by phagocytosis and act as macrophages of the immune system [7]. Figure 3 depicts general images of glia cells. Furthermore, the nervous system is an interconnected and intricate system, where neurons and glia and other cells present communicate with each other *via* numerous pathways and chemicals released called neurotransmitters. This communication is extremely important in the normal functioning of the brain. As this chemical communication slowly degrades, neurodegenerative diseases are born.

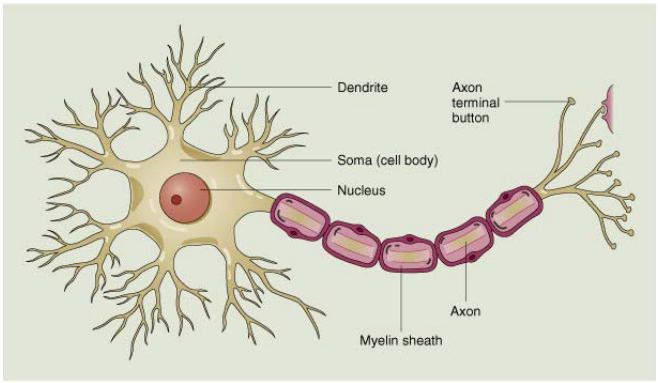

Figure 2. Picture taken from http://www.usm.maine.edu/psy/broida/101/neuron.JPG (accessed on 4/19/05)

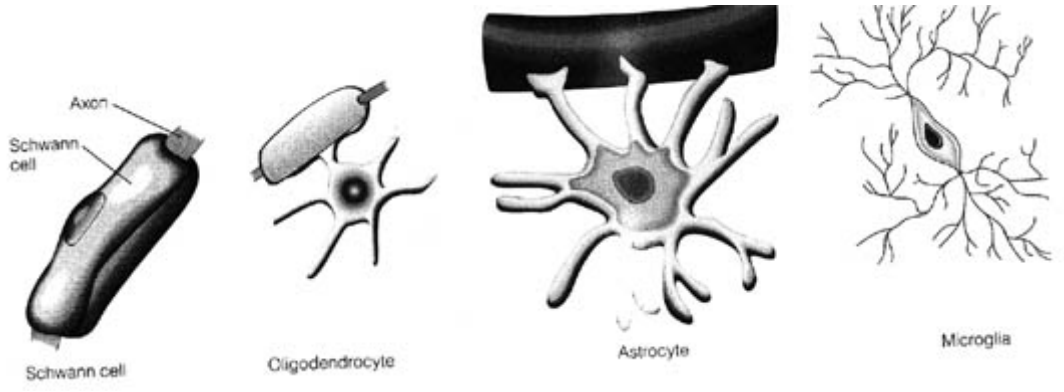

Figure 3. Picture taken from http://homepage.psy.utexas.edu/homepage/class/ (accessed on 4/19/05)

2.1 **Pathology of HIV infection**

There is no direct evidence on the mechanism of the HIV-1 infection of the human brain, but the general agreement is that it does so indirectly through infected monocytes. The paradox lies in the observation that HIV apparently damages and kills neurons by initiating apoptosis, even though it directly infects microglia and macrophages [1]. This suggests that the infection of macrophages and microglia is not enough to suffer so much damage and that some type of augmentation process has to accompany the infection [2].

2.2 **Timeline**

The process of HIV infection of T-cells that has been proposed involves several steps. In the first step monocytes are produced and matured in the bone marrow stimulated by the macrophage colony stimulating factor (M-CSF). These monocytes are released in the blood and are subsequently infected by the HIV-1. They will then start to express CD16 and CD19 which are antigens in early T-cell activation [1-3, 8-9]. After their activation, monocytes migrate and penetrate the blood-brain barrier and begin the recruitment of other monocytes and microglia (that may not be activated). The infected

monocytes and microglia release gp120 viral proteins, which bind to chemokine and cytokine receptors on other microglia [1, 9]. The next phase involves the release of neurotoxins by these infected microglia and macrophages. These neurotoxins include proinflammatory cytokines such as TNF-α and IL-1β, free radicals such as superoxide $O_2^{\bullet -}$ and nitric oxide $NO^{\bullet}$, eicosanoids such as arachidonic acid and PAF (platelet-activating factor) and cysteine that acts as a glutamate agonist [1-3, 8-10]. TNF-α and IL-1β stimulate the growth of astrocytes. The free radicals released, $NO^{\bullet}$ and $O_2^{\bullet -}$, react to produce peroxynitrite. Arachidonic acid inhibits the reuptake of glutamate by astrocytes [1-3, 8-9]. Figure 4 is a general schematic of the HIV-1 infection in the human brain [1]. Ultimately, the cause of neuronal injury in HIV-1 infection is attributed to the overstimulation of NMDA receptors through glutamate binding and influx of Ca^{2+} ions.

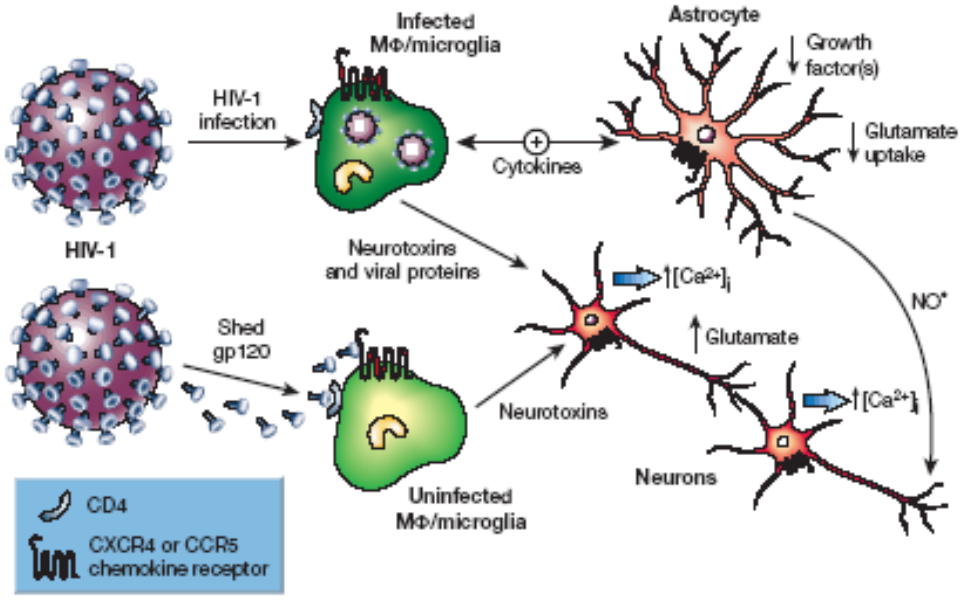

Figure 4. A general schematic of HIV-1 induced neuronal damage. Viral envelope proteins released from HIV bind to chemokine and CD4 receptors in microglia. These microglia start to produce neurotoxins (free radicals, excitatory amino acids such as glutamate and L-cysteine, arachidonic acid, leukocyte and white blood cell activating factors such as PAF and TNF-α, etc). The flux of neurotoxins cause an increase in intracellular Ca^{2+} (possibly by overactivating NMDAR coupled ion channels), which triggers a cascade of free radicals ($NO^{\bullet}$ and $O^{2\bullet -}$), enzymes and more glutamate. This results in neuronal damage. (adapted from [1])

3.1 **NMDA receptors and glutamate**

3.1.1 ***Excitatory amino acids as neurotransmitters***

The first report of an amino acid to exhibit an excitatory effect on neurons was brought forth in 1959 by Curtis and co-workers [11]. They observed that glutamate had a depolarizing effect on rat spinal neurons. Glutamic acid (or glutamate) is not the only substance to display such a behavior. In fact other dicarboxylic acids including aspartic acid as well as quisqualic, kainic and domoic acids or NMDA behave similarly to glutamate [11]. Structures of some excitatory amino acids are shown in figure 5.

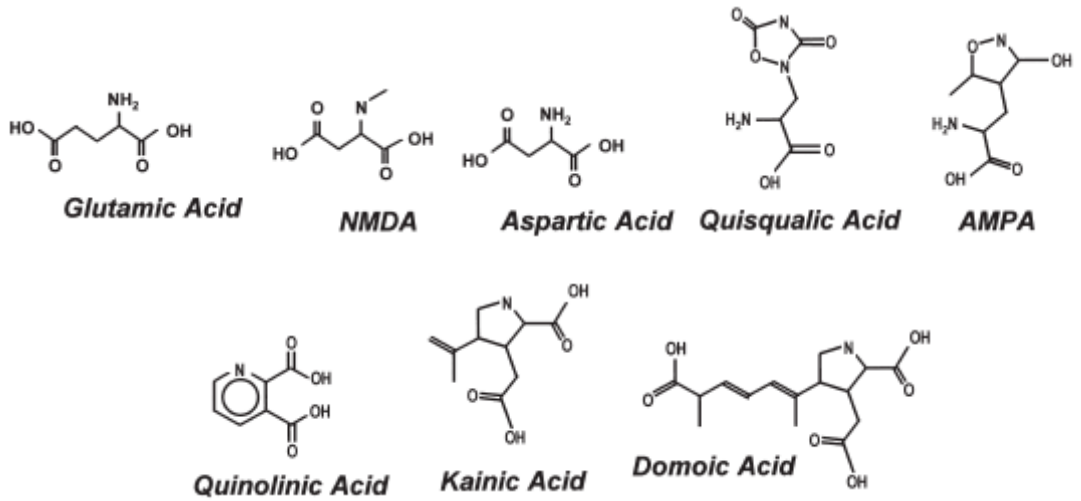

Figure 5. Structures of some common EAAs (excitatory amino acids) Adapted from [11].

One third of all rapid excitatory synapses in the nervous system are attributed to glutamate making it the most important of the EAAs. When glutamatergic nerve terminals are stimulated through depolarization during the normal physiological process of signal transmission, they excrete glutamate extracellularly [11, 12]. Glutamate then crosses the synaptic cleft and stimulates postsynaptic neurons causing depolarization. Action potentials are then generated once the depolarization reaches a certain threshold. Once glutamate fulfills its excitatory role, uptake systems in nerve terminals and glia

cells quickly remove it from the synapse [11]. Specific proteins make certain that glutamate is transported together with sodium ions intracellularly and potassium ions are expelled utilizing the electrochemical gradient created by sodium and potassium [11]. This ensures the uptake of glutamate intracellularly and maintains low extracellular concentrations (glutamate concentration inside the cells is approximately 10,000 times greater than the extracellular one: intracellular glutamate concentrations reach approximately 10 mmol L^{-1}, whereas the extracellular concentrations are approximately 0.6 µmol L^{-1} with 2-5 µmol L^{-1} causing significant damage to neurons) [11, 12].

Glia cells and in particular astrocytes are burdened with the task of keeping the levels of glutamate at a low concentration in the extracellular space. As glutamate is transported into the cell by proteins such as EAAT-1 and EAAT-2 (excitatory amino acid transporter 1 and 2) gets converted to glutamine by an enzyme found in the cytoplasm of astrocytes called glutamine synthase [11]. The newly formed glutamine is subsequently

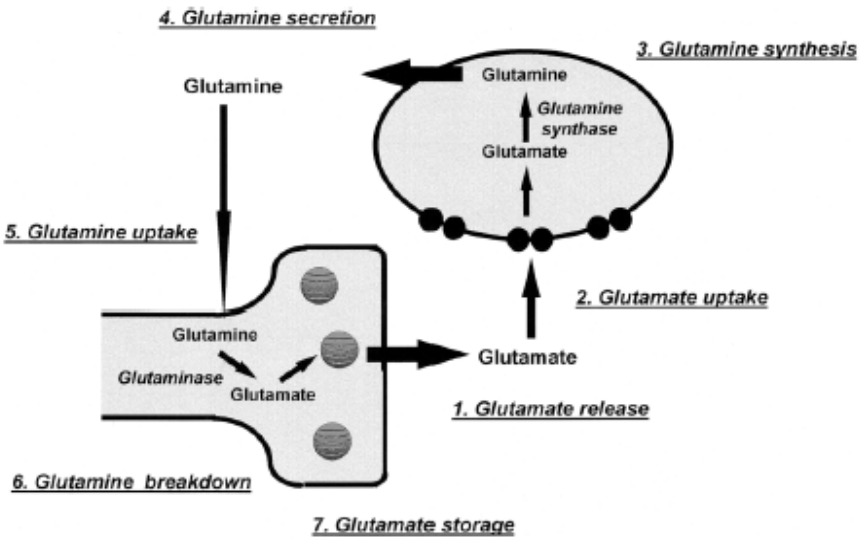

Figure 6. The glutamate cycle. Glutamate is transported into the cell and gets converted to glutamine by glutamine synthase. It then gets released outside the cell only to be picked up by nerve terminals and converted back to glutamate by the enzyme glutamase. Glutamate is then ready to be re-released in another synaptic transmission cycle. Picture taken from [11].

released back into the extracellular space and since it cannot bind to EAA receptors is practically harmless. Nerve terminals take up the extracellular glutamine and convert it back to glutamate *via* the enzyme glutaminase. Glutamate is now ready to be re-released again for new synaptic transmissions [11]. Figure 6 shows a general scheme of the glutamate uptake, release and metabolism in the nervous system (adapted from [11]).

3.1.2 *Excitatory Amino Acid Receptors*

There are three families of EAA receptors: NMDA receptors, AMPA/kainate receptors and metabotropic receptors. NMDA receptors are believed to play a key role in excitotoxicity and hence in neurodegenerative diseases including AIDS dementia (other receptors are also being studied and may play some role in more chronic diseases as well). NMDA and AMPA/kainate receptors are ligand-gated ions channels and use glutamate as an EAA for fast synaptic transmission [11, 13]. Metabotropic receptors are linked to G-proteins. They facilitate slow synaptic transmission and are not believed to play an important role in excitotoxicity and neurodegenerative diseases.

3.1.3 *Structure of NMDA receptors*

Like other ligand-gated ion channel receptors, NMDA receptors are oligomeric proteins composed of an NR1 subunit and at least one NR2 subunit. NR1 cannot be incorporated effectively into the cell membrane without the presence of the NR2 subunit. The NR1 subunit is found in all NMDA receptors and appears to be a crucial component for the normal activity and functioning of these receptors [11, 14]. NR1 subunit is believed to contain the binding site for the cofactor glycine and the glutamate binding site. There are four types of NR2 subunits (A-D) with NR2B being the most abundant in mammalian brains and also believed to be the vital type of the NR2 subunit for suitable functioning of neuronal activity.

3.1.4 ***Function of NMDA receptors***

The primary role of NMDA ligand-gated ion channel receptors is the controlled entry of sodium and calcium ions and the efflux of potassium ions to maintain membrane potentials. They have unitary conductance of 40-50 ps and slow desensitization. At resting membrane potentials these receptors are blocked by magnesium ions, which do not allow the binding of glutamate to the binding site. The blocking mechanism is, however, voltage dependent so once the neuron is depolarized by a different stimulus extracellular glutamate can bind to the binding site of the NMDA receptor, causing an influx of calcium and sodium ions into the neuron [11-12, 14]. Other factors that influence the proper functioning of this receptor are: glycine as a cofactor, extracellular pH (protons bind to this receptor as well), reduction and oxidation of disulfide bridges within the receptor and its phosphorylation state.

Glycine binds to NMDA receptors at a different site than glutamate making it a necessary cofactor. The activation of NMDA receptors is only possible when glycine is bound to its appropriate site in the NR1 subunit of the NMDA receptor. The glycine concentration required for binding this receptor is approximately 0.1 μM, which is well exceeded (~ 0.1 mM) by the concentration of glycine under physiological conditions [11].

4.1 **Excitotoxicity**

Excitotoxicity is the process of prolonged depolarization of neurons, increase of Ca^{2+} intracellularly and the initiation of programmed cell death [11]. The main mechanism for this process has been attributed to NMDA receptors. As mentioned above, NMDA receptors are responsible for the influx of Ca^{2+} into the cells and this increase in concentration is believed to initiate programmed neuronal cell death [11-12,

14]. Three main events are categorized in excitotoxicity: sodium influx, calcium influx and release of intracellular glutamate in the extracellular environment. These processes are interdependent with the early necrotic events attributed to sodium influx, delayed neurodegenerative events to calcium and the actual proliferation and increase of degenerative events to glutamate release.

4.1.1 *Excitotoxicity and voltage dependent sodium channels*

During normal neuronal functioning and signal propagation, the depolarization of neurons is initiated in the AMPA/kainate receptors and thus the activation of voltage dependent sodium channels. The influx of sodium ions causes further depolarization and if the depolarization process becomes chronic enough it causes the NMDA receptors to break free from the magnesium ion block and become susceptible to glutamate activation [11]. The influx of sodium ions disrupts the osmotic balance of the cell and is followed by an influx of chloride ions to maintain the charge balance. This is followed by the entry of water since there is an osmotic gradient, which causes the cell to swell and disruption of organelles due to dilution of the cytoplasm [11, 12]. The cell then lyses and the contents are spilled over into the extracellular environment. This process seems to be reversible with the removal of the depolarizing stimulus. The next step in this cascade, the influx of calcium ions seems to be the most harmful and necessary for the damage caused by excitotoxicity [11].

4.1.2 *Excitotoxicity and calcium ions*

During resting membrane potentials the intracellular concentration of calcium ions is relatively low (approximately 10^{-7} M). This concentration increases *via* two processes: during neuronal depolarization and entry through voltage-dependent calcium channels

and entry through the NMDA receptors whose magnesium block is removed during excessive depolarization [11]. The entry of calcium ions into the cell through NMDA receptor activation is followed by recruitment of more calcium ions from cell storage by unknown mechanisms. This step in excitotoxicity is not reversible by removal of the depolarization stimulus, so the resistance of cells to death caused by excitotoxicity may be dependent on their calcium ion buffering capacity. The influx of calcium ions will eventually elicit the activation of enzymes and different protein-protein interactions that will lead to cell death by disrupting cell homeostasis [11]. Some enzymes that are activated are nucleases, which disintegrate DNA and disrupt chromatin packing, proteases in the cytoplasm, which attack organelles such as the cytoskeleton, kinases such as protein kinase C, which cause the phosphorylation of proteins in the cytoplasm and lipases, which damage the cell membrane causing cell death [11]. A general schematic of calcium dependent excitotoxicity is pictured in figure 7 [11].

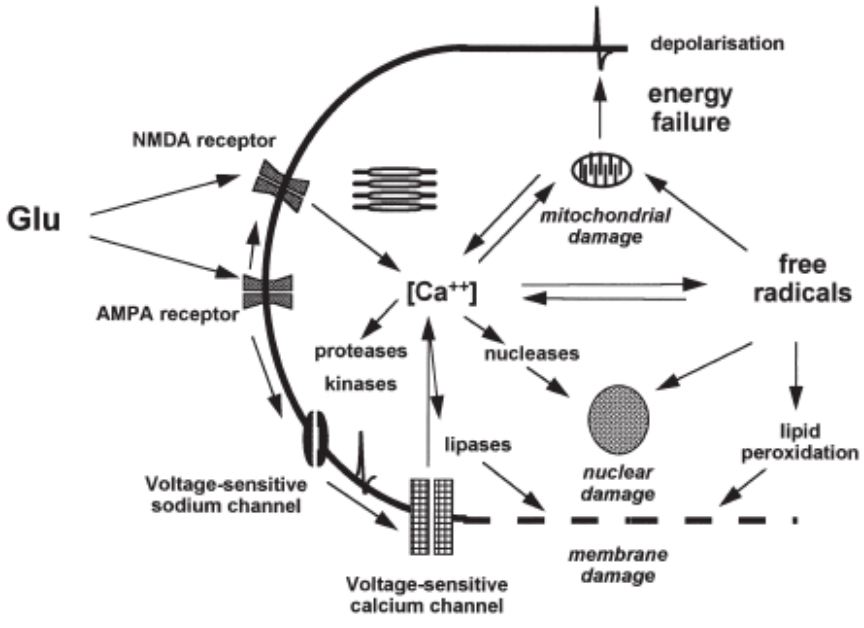

Figure 7. Influx of calcium ions from activation of NMDA and non NMDA receptors and the cascade of reactions triggered inside the cell. Picture taken from [11].

4.1.3 ***Excitotoxicity and free radicals***

Free radicals are generated during excitotoxicity when enzymes such as phospholipase A_2, xanthine oxidase and nitric oxide synthase are activated from the influx of calcium ions[11]. Intracellular phospholipase A_2 requires Ca^{2+} ions to be activated susequently migrating from the cytosol to the membrane for the purpose of catalyzing the breakdown of arachidonate from phospholipids [15]. Studies have shown that Ca^{2+} ion activation of a serine protease modulates the formation of xanthine oxidase from xanthine dehydrogenase. Xanthine oxidase is a well established source of H_2O_2 and superoxide radicals [16]. Production of free radicals from these enzymes is enhanced by the impaired reactions in the electron chain of damaged mitochondria [11, 17]. This overproduction of unchecked free radicals coupled with activated enzymes leads to lipid membrane degradation and nuclear and mitochondrial DNA damage (refer to figure 7). The main free radical implicated in these events is $NO^{\bullet}$, with seemingly two different mechanisms of action.

4.1.4 ***Nitric Oxide mediated excitotoxicity***

$NO^{\bullet}$ (nitric oxide) has been found to wear many hats during *in vivo* studies. This free radical is known to act as a vasodialator (endothelial-derived relaxing factor), a secondary neurotransmitter and a mediator in microglia induced toxicity [18-21]. $NO^{\bullet}$ is produced during normal metabolism *in vivo* by the enzyme nitric oxide synthase (NOS) [22-23]. NOS catalyzes the conversion of L-arginine to L-citrulline with the subsequent release of $NO^{\bullet}$. The excessive stimulation of NMDA receptors triggers the influx of Ca^{2+} ions increasing its intracellular concentration. The Ca^{2+} ions bind to calmodulin, which is a cofactor for the enzyme nNOS causing an increase in nNOS production of $NO^{\bullet}$ [18-19, 24]. Studies have shown that NMDA receptor mediated toxicity is attenuated by the

addition of NOS inhibitors or the removal of L-arginine, implicating $NO^{\bullet}$ in NMDA receptor mediated toxicity [18-19, 24]. There is a proposed dual mechanism of $NO^{\bullet}$ toxicity. Once produced by nNOS, $NO^{\bullet}$ quickly reacts with the superoxide radical ($O_2^{\bullet-}$) forming peroxynitrite (rate of reaction of $O_2^{\bullet-}$ with SOD is 2×10^9 M^{-1} s^{-1} and rate of reaction of $O_2^{\bullet-}$ with $NO^{\bullet}$ is 6-10 x 10^9 M^{-1} s^{-1}) [25-26]. It is noteworthy to mention that purified nNOS produces $O_2^{\bullet-}$ in a calcium/calmodulin dependent manner [19, 24, 27]. Peroxynitrite subsequently breaks down to form hydroxyl radical ($^{\bullet}OH$) and nitroxyl radical ($NO_2^{\bullet}$), which are both very reactive species ($^{\bullet}OH$ being the most oxidizing free radical *in vivo*) [19, 24-25]. The hydroxyl radical will react as soon as it is generated causing DNA damage, lipid peroxidation etc, depending on where it is generated. The other mechanism of $NO^{\bullet}$ toxicity is the activation of guanylate cyclase which mediates the conversion of GTP to cGMP [17, 20]. An increase in cGMP levels causes an increase in Ca^{2+} ions and the mobilization of Ca^{2+} ions from intracellular storage contributing to toxicity. Free radicals and Ca^{2+} ions seem to be involved in a vicious circle, fueling the release of each-other and enhancing neuronal toxicity.

4.1.5 ***Superoxide mediated excitotoxicity.***

Studies show that superoxide is also a factor in free radical-mediated neurotoxicity. Formation of peroxynitrite demands the presence of superoxide in close proximity to the nitric oxide radical. Superoxide radical can be produced *in vivo* through the one-electron reduction of oxygen from complexes I and III of the electron transport chain of the mitochondria [28]. Electrons are passed through to complex III and IV where O_2 is reduced to water, with H^+ ions being pumped out of the mitochondrial membrane [28]. Some $O_2^{\bullet-}$ sources include NADPH oxidase, cyclooxygenases,

lipoxygenases, xanthine oxidase, NOS and the mitochondrial electron chain subunits [25]. Cyclooxygenases and lipoxygenases catalyze the multiple step oxygenation of arachidonic acid and may result in $O_2^{\bullet-}$ production. In ischemia/reperfusion injury, xanthine dehydrogenase gets converted to xanthine oxidase and becomes a source of $O_2^{\bullet-}$. As mentioned earlier, nNOS is a source of $O_2^{\bullet-}$ as well [19, 24, 27]. NADPH oxidase (NOX family of proteins) is probably the main source of $O_2^{\bullet-}$ in activated monocytes and microglia. This enzyme catalyzes the one electron reduction of molecular oxygen to $O_2^{\bullet-}$ in response to a stimulus [29]. This response is quick and the $O_2^{\bullet-}$ production is high. The $O_2^{\bullet-}$ radical is then transported outside the cell and is involved in microbicidal action.

Similar to $NO^{\bullet}$ mediated toxicity, there are two pathways of $O_2^{\bullet-}$ injury during HIV infection. The first pathway involves the observed increase in extracellular $O_2^{\bullet-}$ secreted from infected microglia. This increase in extracellular $O_2^{\bullet-}$ can lead to neuronal membrane lipid peroxidation and cell lysis. The second pathway is closely related to the observed increase in Ca^{2+} levels during excessive glutamate activation of NMDA receptors. This increase in Ca^{2+} ions leads to an increase in autooxidation of ubisemiquinone and NADH-CoQ reductase in the mitochondrial matrix [16], as a consequence, the production of $O_2^{\bullet-}$ goes up. The fate of $O_2^{\bullet-}$ is then decided by the competition of the dismutation reaction and the subsequent formation of H_2O_2 and the reaction of $NO^{\bullet}$ with $O_2^{\bullet-}$ to form peroxynitrite (for rates of reactions see section 4.1.4) [16]. Both of these products can participate in neuronal cell injury. Peroxynitrite can decompose into $NO_2^{\bullet}$ and $^{\bullet}OH$ radicals. Because $^{\bullet}OH$ is a very reactive species, oxidation reactions will immediately be initiated resulting in DNA damage, lipid

peroxidation reactions etc. H_2O_2 is also a potential $^{\bullet}OH$ donor if submitted to Fenton reactions [16]. Even if these radicals only slightly surpass the activity of antioxidant defenses, they can have fatal consequences on neuronal cells if exposed over long periods of time [16].

5.1 ***Experimental proposal***

Since both $O_2^{\bullet-}$ and $NO^{\bullet}$ are involved in neuronal toxicity in AIDS dementia it would be prudent to focus on one of the two species. This experimental proposal will investigate the involvement and extent of $O_2^{\bullet-}$ toxicity in AIDS dementia.

Hypothesis: Increased superoxide production by HIV infected microglia and/or superoxide radical production by neurons themselves is partly responsible for free radical induced damage in neurons of AIDS dementia patients.

Experimental procedure:

- The goal of the first experiment is to determine basal levels of concentration of extracellular $O_2^{\bullet-}$ from microglia/neurons.

  1. Individually culture microglia. One culture will be kept as control and the other cultured with HIV. DMPO spin trap and EPR will be utilized to measure the $O_2^{\bullet-}$ production and compare the wild type culture to the infected microglia.

  2. Individually culture neurons. One culture will be kept as control and the other cultured with HIV. DMPO and EPR will be used to measure the $O_2^{\bullet-}$ production in this experimental sequence. Measurements of DNA damage and lipid peroxidation products will also be monitored throughout the experiments. For measuring DNA damage a measurement of antibodies for 8-oxodG will be

performed and for lipid peroxidation measurements of ethane and pentane gases will be performed.

Measurements of $O_2^{\bullet-}$ production, 8-oxodG and ethane and pentane gases for the first experiment will determine the baseline values against whom all other experimental measurements will be compared to.

- The goal of the second experiment is to determine if there is a statistical difference between concentrations of secreted $O_2^{\bullet-}$ in normal microglia vs. the HIV infected.

  1. Individually culture microglia. One culture will be used as control; one will be cultured with HIV; one with a transfected gene for overexpression of MnSOD and CuZnSOD (to minimize endogenous $O_2^{\bullet-}$) and one with a knockout gene for CuZnSOD and MnSOD that is cultured with HIV.

If this experiment will determine that the $O_2^{\bullet-}$ produced in infected microglia is significantly higher than the amount produced in normal ones, I can proceed to the next experiment.

- The goal of the third experiment is to determine if $O_2^{\bullet-}$ damage comes from the secreted $O_2^{\bullet-}$ from infected microglia or if it endogenous to neurons.

  1. Culture neurons and microglia together. Use one culture as control, culture one with the neurons and HIV infected microglia; one with neurons that overexpress CuZnSOD and MnSOD and HIV infected microglia;one with neurons and HIV infected microglia that are transfected for the overexpression of CuZnSOD and MnSOD.

If this experiment will determine that the $O_2^{\bullet-}$ damage is indeed due to the secreted $O_2^{\bullet-}$ from infected microglia then I can continue with the next experiment.

- The goal of the fourth experiment is to determine that there is neuronal damage caused by the secretion of $O_2^{\bullet-}$ from infected microglia excluding damage caused by the influx of Ca^{2+} ions into neurons.

  1. Culture neurons and microglia together. Use one culture as control; one with neurons and HIV infected microglia; one with neurons and CuZnSOD/MnSOD transfected microglia; one with neurons and infected microglia with a metal chelator to get rid of extracellular Ca^{2+} ions and the last one with neurons, a Ca^{2+} ion chelator and CuZnSOD/MnSOD transfected microglia.

If this experiment determines that there is substantial neuronal damage caused by infected microglia (excluding damage from Ca^{2+} ion damage) then I can continue with the next experiment.

- The goal of the fifth experiment is to determine that there is neuronal damage caused by the secretion of $O_2^{\bullet-}$ from infected microglia excluding damage caused by excessive $NO^{\bullet}$ radical production.

  1. Culture neurons and microglia together. Use one culture as control; one with neurons and infected microglia with 1400W (an inhibitor of iNOS) and a Ca^{2+} chelator; one with neurons and infected miocroglia both overexpressing CuZnSOD/MnSOD with 1400W a Ca^{2+} chelator; one with neurons overexpressing CuZnSOD/MnSOD and infected microglia with a Ca^{2+} chelator and with 1400W (an inhibitor of iNOS).

If this experiment determines that there is neuronal damage observed even after blocking the production of $NO^{\bullet}$, then my hypothesis will be proven.

Some potential problems that may arise using EPR and spin trapping for some of these experiments may be the differentiation between intra and extracellular sources of $O_2^{\bullet -}$ (spin traps work better for extracellular $O_2^{\bullet -}$). Some other problems may arise from measuring DNA damage through antibodies of 8-oxodG since DNA damage may not be due only to $O_2^{\bullet -}$ damage. The same argument is true for measuring the by products of lipid peroxidation (ethane and pentane).

6.1 *Future work*

A strong emphasis is placed on finding treatments that would improve the conditions of AIDS dementia patients. Antiretroviral therapy has proven to be worthy of stopping the HIV infection in the body, however these drugs cannot cross the blood-brain barrier. This means that the infection finds a safe haven in the brain even if it can be contained everywhere else in the body. The continuum of the infection in the brain still leads to the development of AIDS dementia and the slow death of the patients. This makes the discovery and manufacture of either natural or synthetic agents that will be able to cross the blood-brain barrier and be able to stop the mechanism of HIV infection in the brain, of utmost priority. This process is however, closely dependent on the complete understanding of the intricacy of the mechanism of HIV infection in the brain which still remains to be completely elucidated.

7.1 *Conclusion*

AIDS dementia is a form of dementia that is observed in patients who suffer from HIV infection. At this point there is no known cure for this disease. Much effort is being placed on elucidating the inner workings of the mechanism of the HIV infection in the

brain. What is known is that the microglia are infected and the neurotoxins that they produce in response to this infection trigger a cascade of events in neighboring neurons which lead to cell death. Understanding the routes and mechanisms of toxicity from these excreted neurotoxins fuels the hope of finding a cure for this disease in the future.

References

1. Kaul M, Garden G, Lipton S. 2001. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* 410: 988
2. Lipton S. 1998. Neuronal injury associated with HIV-1: approaches to treatment. *Annu RevPharmacToxic.* 38: 159
3. Lipton S, Gendelman H. 1995. Dementia associated with the acquired immunodeficiency syndrome. *N E J Med.* 332: 934
4. Gao F, Bailes E, Robertson D, Chen Y, Rodenburg C, et al. 1999. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes. Nature* 397: 436
5. Bailes E et al. 2003. Hybrid origin of SIV in chimpanzees. *Science* 300: 1713
6. Zhu T, Korber B, Nahmias A, Hooper E, Sharp P, Ho D. 1998. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. *Nature* 391: 594
7. Bear MF CB, Paradiso MA. 2001. *Neuroscience: Exploring the brain.* Baltimore: Lippincott Williams & Wilkins
8. Gartner S. 2000. HIV infection and dementia. *Science* 287: 602
9. Kaul M, Zheng J, Okamoto S, Gendelman H, Lipton S. 2005. HIV-1 infection and AIDS: consequences for the central nervous system. *Cell Death and Diff*: 1
10. Viviani B, Corsini E, Binaglia M, Galli C, Marinovich M. 2001. Reactive oxygen species generated by glia are responsible for neuron death induced by human immunodeficiency virus-glycoprotein 120 *in vitro*. *J Neurosc.* 107: 51
11. Doble A. 1999. The role of excitotoxicity in neurodegenerative disease: implications for therapy. *Pharmac Ther.* 81: 163
12. Lipton S, Rosenberg P. 1994. Excitatory amino acids as a final common pathway for neurologic disorders. *N E J Med.* 330: 613
13. Gasic G, Hollmann M. 1992. Molecular neurobiology of glutamate receptors. *Annu RevPhys.* 54: 507
14. Kaul M, Lipton S. 2000. The NMDA receptor-its role in neuronal apoptosis and HIV-associated dementia. *NeuroAids* 3
15. Dennis E. 2000. Phospholipase A2 in Eicosanoid Generation. *Am J Respir Crit Care Med.* 161: 32
16. Dykens J. 1994. Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated Ca^{2+} and Na^{+}: implications for neurodegeneration. *J Neurochem.* 63: 584
17. Bolanos J, Almeida A, Stewart V, Peuchen S, Land J, et al. 1997. Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J Neurochem.* 68: 2227
18. Dawson V, Dawson T, London E, Bredt D. 1991. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci. USA* 88: 6368
19. Pou S, Pou W, Bredt D, Snyder S, Rosen G. 1992. Generation of superoxide by purified brain nitric oxide synthase. *JBiol Chem.* 267: 24173

20. Leong S, Ruan R, Zhang Z. 2002. A critical assessment of the neurodestructive and neuroprotective effects of nitric oxide. *Ann N Y Acad Sci.* 962: 161
21. Espey M, Miranda K, Thomas D, Xavier S, Citrin D, et al. 2002. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen species. *Ann. N. Y. Acad. Sci.* 962: 195
22. Liu B, Gao H, Wang J, Jeohn G, Cooper C, Hong J. 2002. Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann N Y Acad Sci.* 962: 318
23. Mollace V, Nottet H, Clayette P, Turco M, Muscoli C, et al. 2001. Oxidative stress and neuroAIDS: triggers, modulators and novel antioxidants. *Tre Neurosci.* 24: 411
24. Dawson V, Dawson T, Bartley D, Uhl G, Snyder S. 1993. Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *J Neurosci.* 13: 2651
25. Estevez A, Jordan J. 2002. Nitric oxide and superoxide, a deadly cocktail. *Ann N Y Acad Sci.* 962: 207
26. Beckman J, Beckman T, Chen J, Marshall P, Freeman B. 1990. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci. USA* 87: 1620
27. Kimura T, Kameoka M, Ikuta K. 1993. Amplification of superoxide anion generation in phagocytic cells by HIV-1 infection. *FEBS.*326: 232
28. Brand M, Affourtit C, Esteves T, Green K, Lambert A, et al. 2004. The powerhouse takes control of the cell: the role of mitochondria in signal transduction. *Free Rad Biol Med.* 37: 755
29. Morel F, Doussiere J, Vignais PV. 1991. The superoxide-generating oxidase of phagocytic cells. Physiological, molecular and pathological aspects. *Eur J Biochem.* 201: 523-546.
30. Forman H, Torres M. 2002. Reactive oxygen species and cell signaling Respiratory burst in macrophage signaling. *Am JRespir Crit Care Med* 166: S4