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# The Role of Human Immunodeficiency Virus Type 1 (HIV-1) Proteins and Antiretroviral Drug Therapy in HIV-1-Induced Oxidative Stress

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## 1. Introduction

Over 33 million people worldwide are infected with human immunodeficiency virus type 1 (HIV-1). In addition, over 2.7 million new cases are diagnosed each year with half of these infections occurring in individuals younger than 25 years (UNAIDS, 2008). Fortunately, since the emergence of highly active antiretroviral therapy (HAART) in 1996, morbidity and mortality associated with HIV-1 infection have been markedly decreased. HIV-1 infected patients have demonstrated dramatic decreases in viral burden and opportunistic infections, and an overall increase in life expectancy. Despite the positive HAART-associations outcomes, including the improvement of the clinical course, prognosis, and survival of patients infected with HIV-1, it has become increasingly clear that HIV-1 infected patients have an enhanced risk for developing noninfectious consequences of HIV-1 infection over time. In the last few years, lipodystrophy, characterized by redistribution of body fat, and insulin resistance, have been reported in many HIV-1 infected patients, and their relationship with antiretroviral drugs and HIV-1 infection *per se* have become a subject of debate and researches worldwide. Evidence suggests that HIV-1 infected patients are under chronic oxidative stress that may be involved in the development and progression of the disease. Oxidative stress is enhanced by the chronic inflammation that is associated with activation of lymphocytes and phagocytes, and is accompanied by the direct or indirect effects of several opportunistic pathogens. In addition, HIV-1 proteins and various components of current HAART regimes contribute to oxidative stress-induced disturbances such as cardiovascular disease (including metabolic syndrome and endothelial dysfunction), neurological disorders (HIV-1 dementia), and ocular complications (retinopathy). Cardiovascular complications are been recognized with increasing frequency and are associated with the greatest risk of death in HIV-1 patients. Studies demonstrated that not only do various components of HAART contribute to endothelial cell damage and vascular dysfunction in patients, but also the viral proteins themselves increase cardiovascular risk. HIV-1-associated cardiovascular disease progression is thus most likely a multifactorial process, resulting from a combination of distinct HIV-1 proteins as well as various components of current multidrug antiretroviral therapy (Kline et al., 2008).

It is estimated that one-third of adults infected with HIV-1 develop dementia (Janssen et al., 1992). It was reported that oxidative stress has been demonstrated in the brain and cerebrospinal fluid (CSF) from HIV-1 infected individuals, showing important implications for therapeutic approaches for HIV-1-induced dementia (HIVD).
The aim of this chapter is to review the roles of both HIV-1 proteins and antiretroviral drugs in the development of oxidative stress-induced disturbances such as cardiovascular disease and neurological disorders. For this purpose, studies, *in vitro* and *in vivo*, were identified by a systematic search through PubMed for English-language literature, included original and review articles published up to 2011.

## 2. Oxidative stress and biomarkers

Oxidative stress is defined as an imbalance between the antioxidant and pro-oxidant systems with the shift towards the pro-oxidant system. Oxidative stress is also defined as the modification and accumulation of biological molecules altered by various kinds of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS affect gene transcription and cell growth/proliferation, and they have been considered intercellular signal molecules.
ROS and RNS are highly reactive, toxic oxygen or nitrogen moieties, respectively, such hydroxyl radical, peroxyl radical, superoxide anion, hydrogen peroxide, nitric oxid (NO), and peroxynitrite. The half-life of ROS species varies from nanoseconds for the hydroxyl radical to seconds for NO and peroxyl radicals. Because of the differences in half-lives, the ROS reactivity differs from the aqueous environment in which they were formed to reacting deep within the membrane (Pocernich et al., 2005).
In biological systems, the cellular membrane constitutes in a main target of the ROS and RNS. In addition to the cellular membrane, other intracellular membranes are important target of the oxidative stress such as mitochondrial, nuclear and endoplasmic reticulum membranes that can suffer the lesive action of the ROS and RNS by changing their form and function. Not only enzymes but also receptors and transport proteins can be important early targets of oxidative damage. While most ROS do not diffuse more than a few femtometres (fm), the lipid peroxides that are resulted from the ROS-induced peroxidation of membrane phospholipids, such as malondialdehyde (MDA), can transverse the circulation and cell membranes, with resultant dysfunction of vital cellular processes including membrane transport and mitochondrial respiration (Haliwell, 1987).
ROS can attack double bonds in polyunsaturated fatty acids (PUFAs), inducing lipid peroxidation (LPO), which may result in more oxidative cellular damage. LPO has been defined as the oxidative deterioration of polyunsaturated lipids and its measurement is a laboratorial approach for determining oxidative stress. Peroxides and aldehydes generated are not only passive biomarkers of oxidative stress, but also cytotoxic products (Zwart et al., 1999).
MDA is a three carbon, low molecular weight aldehyde that can be produced from free radicals that attack on PUFAs of biological membranes. The determination of MDA is used for monitoring LPO in biological samples. LPO has been the focus of attention in recent researches because it was commonly thought that the thiobarbituric acid (TBA) test, the commonest assay of LPO *in vitro*, measures free MDA. It arises largely from peroxidation of PUFAs with more than two double bonds, such as linolenic, arachidonic and docosahexaenoic acids. MDA can also be formed enzimatically during eicosanoid

metabolism. Under physiological conditions, proteins are more readily attacked by MDA than are free amino acids, resulting in modification of several residues, especially lysine, as well as intra- and intermolecular protein cross-links.

One particular class of toxic products of LPO is the isoprostanes, a series of prostaglandin-like compounds formed during peroxidation of arachidonic acid. Because they are structurally similar to prostaglandin F2α, isoprostanes are collectively referred as F2-isoprostane. F2-isoprostane is useful marker of LPO and can be measured in human plasma and urine (Halliwell & Gutteridge, 1999).

Collectively, ROS can lead to oxidation of proteins, and DNA, peroxidation of lipids, and ultimately cell death (Butterfield et al., 2001). These protein carbonyl moieties result from a direct oxidation of many amino acids such as lysine, arginine, histidine, proline and threonine, β-scission of the peptide backbone, or from binding of the LPO product 4-hydroxy-2-nonenal (HNE) to proteins. Alterations in proteins can lead to aggregation, changes in secondary and tertiary structure, susceptibility to proteolysis, fragmentation, and loss-of function. LPO produces large amounts of aldehydes, such as HNE, MDA, and acrolein, and leads to isoprostanes formation (Butterfield et. al, 2002). HNE and acrolein contribute to membrane damage and cell death induced by various oxidative insults, and through alterations of protein structure, these molecules are capable of inhibiting DNA, RNA, and protein synthesis, glycolysis, and degradation of enzymes (Pocernick et al., 2005).

ROS produce a multiplicity of change in proteins, including oxidation of –SH groups, hydroxylation of thyrosine and phenylalanine, conversion of methionine to its sulphoxide and generation of protein peroxides. Several assays for damage to specific amino acid residues in proteins have been developed and can be used to assess steady-state levels of oxidative protein damage *in vivo*. The carbonyl assay is a general approach for evaluating oxidation protein damage. It is based on the fact that several ROS attack amino acid residues in proteins that results products with carbonyl groups, which can be measured after reaction with 2,4-dinitrophenylhydrazine (Halliwell & Gutteridge, 1999). Oxidative stress also increases the levels of protein oxidation measured by the Advanced Oxidation Protein Products (AOPPs). AOPPs are novel biomarkers of oxidative damage and are considered as reliable markers to estimate de degree of oxidant-mediated protein damage. AOPPs resulted from the interaction between oxidants and plasma proteins with the oxidation of amino acid residues such as tyrosine, leading to the formation of dityrosine-containing protein cross-linking products detected by spectrophotometry (Witko-Sarsat et al., 1998). Neutrophils that constitute the most important source of chlorined oxidants due to their high content in myeloperoxidase might be involved in plasma AOPPs formation. *In vivo* plasma levels of AOPPs closely correlate with level of dityrosine, a hallmark of oxidized proteins, and with pentosidine, a marker of protein glycation closely related with oxidative stress (Witko-Sarsat et al., 1998).

### 2.1 NO and HIV-1 infection

NO is a free-radical gas, a diffusible messenger that displays a variety of physiological functions, including vasorelaxation, bronchodilatation, inhibition of platelet aggregation, and neurotransmission (Radi, 2004). Additionally, it appears to be involved in the macrophage-dependent killing of intracellular parasites and functions as a tumoricidal and antimicrobial molecule i*n vitro* and *in vivo* (Torre et al., 2002). NO represents an important

component of the host immune response against DNA and RNA viral infections, including HIV-1 infection (Mannick et al., 1995).

NO is synthesized by the family of enzymes called nitric oxide synthase (NOS). Various isoenzymes of NOS, such as endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS) are localized in endothelium, macrophages, and the brain, respectively. In normal endothelial cells, the amino acid L-arginine is constitutively converted to L-citrulline and NO by eNOS.

The iNOS expression is increased by oxidative stress or pro-inflammatory cytokines (Nathan, 1997). However, interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interferon alfa-2b (IFN-α2b), interferon gamma (IFN-γ), and interleukin 17 (IL-17) induce iNOS, whereas transforming growth factor beta (TGF-β), interleukin 4 (IL-4), interleukin 10 (IL-10), interleukin 11 (IL-11), and interleukin 13 (IL-13) suppress the induction of NO released from macrophages (Torre et al., 2002). In addition, HIV-1 also stimulates NO production by human macrophages, inasmuch as concentration of recombinant gp120 HIV-1 envelope glycoprotein *in vitro* increases production of NO by human monocyte-derived macrophages (Pietraforte et al., 1994).

The excessive production of NO by iNOS may contribute to tissue damage in several inflammatory and infectious diseases and this damage may be the price to pay for equipping so many host cells with the ability to deploy this compound against infections. Although NO production can be increased by the iNOS, the biodisponibility of NO can be impaired because NO is consumed in a reaction with superoxide anion yielding a strong oxidant species, the peroxynitrite ($ONOO^{-}$), which in turn accelerates the LPO reaction (Li et al., 2007; Tao et al., 2007). Peroxynitrite production is also supported by the elevated levels of nitrotyrosine, a marker of endogenous peroxynitrite generation found in both human and animal models (Yamaguchi et al., 2006).

Since NO is a very labile free radical with a half-life of only a few seconds and is rapidly oxidized by tissue oxygen to the stable end products, nitrite ($NO_2^-$) and nitrate ($NO_3^-$), it is difficult to measure NO levels in the tissue directly with real time. NO can be evaluated by several methods, including the assessment of NO metabolite (NOx) levels. Commonly, serum NO levels are assessed on the basis of nitrite and nitrate concentration according to the Griess reaction supplemented by the enzymatic reduction of nitrate to nitrite with cadmium (Guevara et al., 1998; Navarro-Gonzales et al., 1998). Following up the changes in nitrite/nitrate levels in the human tissues and plasma samples can be an important tool in understanding NO involvement.

Although NO is an important mediator of the immune response against microorganisms, NO that is produced during the infectious diseases may be also deleterious, particularly in HIV-1 infection where may contribute to AIDS pathogenesis by enhancing viral replication in lymphocytes (Jimenez et al., 2001) and monocytes (Blond et al., 2000), increasing lymphocyte apoptosis (Mossalayi et al., 1999), and participating in the pathogenesis of AIDS-related dementia complex (Adamson et al., 1996). A study demonstrated impaired iNOS mRNA expression and NO levels in peripheral blood mononuclear cells from HIV-1 infected patients, either *in vivo* or *in vitro* HIV-1 infection of normal cells (Cairoli et al., 2008). Low levels of NO have been implicated in lymphocyte activation and proliferation (Barbul et al., 1990). NO donors such as sodium nitroprusside and to a lesser degree gaseous NO, increase lymphocyte uptake of glucose (an early event during lymphocyte activation), stimulate TNF-α production and the transcriptional nuclear factor kappa beta (NF-kB)

binding activity, and enhance activity of tyrosine kinase, p56, which is implicated in lymphocyte signaling events (Lander et al., 1993). Paradoxically, high concentrations of NO, which occur following macrophage activation, suppress antigen presenting cell activity and T cell proliferation (Holt et al., 1991).

In addition, vascular dysfunction and damage have been shown to be associated with impaired endothelial NO metabolism and function. Therefore, iNOS-derived NO mediates the inflammatory response and has been shown to cause vascular dysfunction in a number of experimental models (Gunnett et al., 2003).

The data of NO levels obtained in HIV-1 infected individual samples are controversial. Groeneveld et al. (1996) have shown that serum nitrate concentrations are higher in asymptomatic HIV-1 infected patients than in healthy individuals. In addition, increased production of NO was correlated with RNA-HIV-1 viral load and activation of mononuclear phagocytes in HIV-1 infected patients. Torre et al. (1996a, 1996b) have shown that NO production is increased in AIDS patients with opportunistic infection, whereas nitrite concentrations were normal in asymptomatic patients. These authors have also confirmed increased production of NO and IL-1β, TNF-α, and IFN-γ in the sera of children with HIV-1 infection and they postulated that the increase in the concentration of these cytokines may represent a substantial stimulation of NO production. Zangerle et al. (1995) noted high nitrite and nitrate concentrations in 39 patients with AIDS without opportunistic infections, especially in those with lower $CD4^+$ T cell counts, whereas in asymptomatic patients no such increase was seen. However, a previous study showed no altered endogenous nitrate formation in eight patients with AIDS, most of whom had opportunistic infections (Evans et al., 1994).

However, some aspects must be taken in to account when these apparent controversial results are discussed including the fact that the oxidative stress was evaluated in HIV-1 infected individuals that differed in the clinical course of the disease and in the presence or absence of opportunistic infections. Increases in the NO production may not be observed due the consume resources by the oxidative stress. Anyway, further studies may be necessary to confirm these previous results.

### 2.2 Antioxidants

To neutralize the damaging oxidative stress, natural antioxidant systems have evolved, including enzymes like glutathione (GSH) peroxidase, glutathione reductase, glutathione transferase, superoxide dismutase (SOD), S-methyl transferase, and catalase. Protection against free radicals can also come from small non-protein, cellular antioxidants, nonenzymatic, such as vitamin C, vitamin E, carotenoids, flavonoids, thioredoxin, and uric acid (Butterfield, et al., 1997).

GSH is a tripeptide (γ glutamate-cysteine-glycine) present in high concentrations in all mammalian cells that has many critical protective and metabolic functions. GSH detoxifies electrophilic metabolites of xenobiotics and protects cells from the toxic effects of free radicals and ROS (Bleuter, 1989). It is also important in the immune response against infections and plays an important role in lymphocyte proliferation, antibody-dependent and cell-mediated cytotoxicity, and protection of lymphocytes against superoxides that are produced to destroy invading pathogens (Droge et al., 1991; Smyth, 1991). N-acetyl-L-cysteine (NAC) acts as an indirect precursor of GSH by raising levels of cysteine, a precursor of GSH. Whey proteins have been shown to increase GSH levels in human, most likely by

supplying the amino acid cysteine necessary for the synthesis of GSH (Pocernich et al., 2005).

Vitamin C represents the major water-soluble antioxidant in the human body. Ascorbate protects cell components from free radical damage by quenching water soluble radicals, scavenging lipid-peroxidation-derived radicals, or reducing tocopherol radical to tocopherol (Stehbens, 2004).

SOD is an endogenous antioxidant that catalyses de dismutation of the superoxide anion radical (Stambullian et al., 2007).

Vitamin E, a potent chain breaking lipid soluble antioxidant, reacts with lipid peroxyl radical eventually by terminating the peroxidation chain reaction and thereby by reducing oxidative damage. Vitamin E acts as an antioxidant on biomembranes and it is the principal lipid soluble chain-breaking antioxidant in mitochondria, microsomes, and lipoproteins.

Selenium is an essential nonmetal trace element that is necessary for normal immune function. Selenium also increases the GSH peroxidase activity and its deficiency diminishes cell-mediated immunity and depresses B-cell function (Stehbens, 2004).

## 3. Evidences of oxidative stress in individuals infected with HIV-1

The hallmark of HIV-1 infection is the cellular $CD4^+$ T cell immunodeficiency; however, the real cause of the loss of these cells is unknown. The most widely accepted hypothesis is that HIV-1 primes the cell to apoptotic death. Different agents appear to trigger apoptosis in $CD4^+$ T cells, including viral protein, inappropriate secretion of inflammatory cytokines by activated macrophages and toxins produced by opportunistic microorganisms. Since oxidative stress can also induce apoptosis, it can be hypothesized that it could participate in $CD4^+$ T cell apoptosis observed in AIDS (Reppeto et al., 1996).

Evidence suggested that HIV-1 infected patients are under chronic oxidative stress. This effect is subsequent to depletion of endogenous antioxidant moieties and to an increased production of ROS. Observation of the multiple pathogenic interactions between ROS and the HIV-1 has drawn attention to the possibility that these types of the interaction may play a role in the pathogenesis of many other viruses as well. ROS has been suggested to be involved in many aspects of HIV-1 disease pathogenesis, including increase viral replication, inflammatory response, decrease of immune cell proliferation, loss of immune function, chronic weight loss, and increase sensitivity to drug toxicity. In addition, antiretroviral combination therapy increases protein oxidation as well as the level of oxidative stress already present in HIV-1 infection (Ngondi et al., 2006).

One aspect of the role of ROS in HIV-1 pathogenesis is the positive modulatory effect on the immune activation, important both in eradication of viral infection but also in immune-induced cellular injury (Schwarz, 1996). HIV-1 infections causes a chronic inflammation as shown by high plasma levels of pro and inflammatory cytokines, chemokines and ROS in seropositive individuals (Israel & Gougerot-Pocidalo, 1997). Increased production of ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide may be related to an increased activation of polymorphonuclear leukocytes during HIV-1 infection or influenced by the pro-oxidant effect of pro-inflammatory cytokines produced by activated macrophages during the course of HIV-1 infection (Das et al., 1990)

In HIV-1 infected patients, the increased oxidative stress has been implicated in increased HIV-1 transcription through the activation of NF-kB. NF-kB is bound to kinase inhibitor nuclear factor-kB (IkB) in the cytoplasm in its active form, but various factors, such as TNF-

α and ROS, can cause the release of NF-kB from factor IkB, and NF-kB translocates to the nucleus and binds to DNA. In this way, the NF-kB is available to bind in the nuclear DNA and to induce HIV-1 gene transcription (Schereck et al., 1991). Thus, oxidative stress may potentially be involved in the pathogenesis of HIV-1 infection through direct effects of cells and through interactions with NF-kB and activation of HIV-1 replication (Greenspan & Aruoma, 1994; Israel & Gougerot-Pocidalo, 1997).

The activation of phagocytes induced by HIV-1 is associated with oxidative stress, not only because ROS are released but also the fact that activated phagocytes may release pro-oxidant cytokines, such as TNF-α and IL-1, which promote iron uptake by the monocyte macrophage system. TNF-α is synthesized in infected host cells, produces pro-oxidant effects in mitochondria, and inhibits mitochondrial respiration at Site II, the site of superoxide production (Schulze-Osthoff et al., 1992). Other cytokine that is involved in the oxidative stress is the IL-1. Activated monocytes produce IL-1 that stimulates neutrophils to release lysosomal proteins, including lactoferrin. This protein rapidly binds iron and this complex accumulates in the monocyte macrophage system. If the accumulated iron exceeds cellular iron-binding capacity, unbound pro-oxidant iron could interact with the superoxide via Fenton's reaction and produces hydroxyl radicals (Halliwell, 1987).

Oxidative stress biomarkers (pro-oxidants and antioxidants) have been investigated in HIV-1 patients serum samples; however, previous studies show inconsistent findings regarding MDA levels in these patients. One study showed significantly elevated serum MDA concentration in HIV-1 infected patients, where HIV-1 symptomatic presented higher levels than asymptomatic patients, suggesting that the infection results in oxidative stress of the host lipids (Sönnerborg et al., 1988; Jordão Júnior et al. 1998; Suresh et al., 2009). The oxidative stress was evaluated by the LPO and GSH plasma levels in 150 HIV-1 infected individuals and in 30 healthy controls, and the results showed that the mean LPO plasma levels were significantly higher in HIV-1 infected individuals as compared to healthy controls, and the mean GSH level in HIV-1 infected individuals was significantly lower compared to healthy controls. In addition, there was a significant positive correlation between absolute $CD4^+$ T cells and GSH levels. However, there was no significant difference in the levels of LPO and GSH among HIV-1 infected individuals receiving antiretroviral therapy (ART) and those without ART (Wanchu et al., 2009).

Jordão Júnior et al. (1998) evaluated 28 serologically positive HIV-1 patients, 16 patients with AIDS (with < 200/$mm^3$ $CD4^+$T lymphocytes) and 12 HIV-1 infected and asymptomatic patients (with 200-500/$mm^3$ $CD4^+$ T lymphocytes). The control group consisted of 11 healthy individuals. All individuals showed normal plasma vitamin A levels. However, urinary excretion of vitamin A and MDA was higher in AIDS patients than in HIV-1 asymptomatic patients and considerably higher than in the control subjects. Therefore, the severe oxidative stress that occurs in the HIV-1 seropositive patients in comparison with seronegative individuals can exert a role in the progression of disease (Suresh et al., 2009)

## 4. Oxidative stress and cardiovascular diseases associated with HIV-1 infection

Endothelium dysfunction is an initial step in the development of cardiovascular diseases, especially atherosclerosis, and is associated with an increase in oxidative stress. HIV-1 infection is associated with increased ROS production and chronic oxidative stress,

suggesting a role of ROS in HV-1-induced endothelial cells dysfunction. Evidence from experimental, observational, and clinical studies suggests that HIV-1 infection itself and the associated pro-inflammatory response can increase the risk of cardiovascular disease.

Multiple mechanisms, both specific and overlapping ways, are proposed to explain how HIV-1 proteins damage the endothelium, considering that viral genome contains nine main genes (*gag, pol, env, tat, rev, vpu, vpr, vif,* and *nef*) and encodes for approximately 15 mature HIV-1 proteins that may interact with any number of unique targets. Proteolytic cleavage of the Gag-Pol precursor protein yields the major structural components of the viral core including matrix p17, capsid p24, nucleocapsides p9 and p6, reverse transcriptase (RT), protease and integrase. Proteolytic cleavage of Env produces the important envelope glycoprotein (gp) gp120 and gp41. The remaining genes encode for the regulatory proteins Tat and Rev, and the accessory proteins Vpu, Vpr, Vif, and Nef (Greene, 1991).

The gp120, Tat, Vpu, and Nef proteins exert some important effects on endothelial cell homeostasis (reviewed by Kline & Sutliff, 2008). HIV-1 proteins can activate several inflammatory pathways in the vascular wall with cytokines release and expression of endothelial molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and E-selectin (Seigneur et al., 1997; Greenwood et al., 1998; Wolf et al. 2002). The gp120 increases the expression of ICAM-1, but not VCAM-1 or E-selectin, in human coronary artery, lung, brain, umbilical vein, and dermal microvascular endothelial cells. The gp120 also induces the apoptosis in human coronary endothelial cells, the adhesion of monocytes and lymphocytes to the endothelium; gp120 increases the endothelium permeability through cytoskeletal rearrangement, downregulation of tight junction proteins, and increases ROS production. The gp120 negatively affects endothelium function through the production of potent vasoconstrictors. The nonstructural Tat protein contains 86-101 amino acids that are formed from two exons. The first exon contributes to the first 72 amino acids and acts as a transacting nuclear regulatory protein actively secreted by infected cells that is essential for viral replication.

Similar to gp120, Tat protein can promote the apoptosis, monocyte chemoattraction and adhesion, endothelium permeability, proliferation, angiogenesis, and an increase in the expression of matrix metalloproteinases and ROS. It has been demonstrated that viral Tat protein liberated by HIV-1 infected cells interferes with calcium homeostasis, activates caspases and induces mitochondrial generation and accumulation of ROS, all being important events in the apoptotic cascade of several cell types. When activated, peripheral blood T lymphocytes are induced to express Fas/APO-1/CD95 receptor that mediates apoptosis when binding to Fas ligand. $CD4^+$ T cell subset depletion in HIV-1/AIDS patients is the most dramatic effect of apoptosis mediated by redox abnormalities and induction of Fas/APO-1/CD95 receptor expression (Westendorp et al., 1995; Kruman et al., 1998; Jaworowski & Crowe, 1999).

In patients with uncontrolled HIV-1 infection, vasculitis are also observed in small blood vessels, aneurysms in medium and large arteries, significantly decreased levels of high-density lipoprotein (HDL) cholesterol, and elevated plasma levels of von Willebrand factor, plasminogen activator inhibitor-1 (PAI-1) antigen, and tissue-type plasminogen activator (tPA). Although HIV-1 is likely not vasculotropic, the virus affects endothelium homeostasis and function in important ways (Kline & Sutliff, 2008).

The vascular endothelium is exposed continually to a number of viral stimuli in the bloodstream. These stimuli include: a) HIV-1 infected $CD4^+$ T cells, monocytes, and macrophages; b) freely circulating HIV-1 viruses; c) HIV-1 proteins released upon host cell lysis; d) actively secreted proteins (Tat and gp120); and e) viral-induced pro-inflammatory cytokines. HIV-1-induced cytokines may also activate the endothelium, leading to enhanced production of ROS, and the release of chemoattractant at localized areas of vascular inflammation. HIV-1-infected individuals have higher plasma levels of hydroperoxides and MDA compared with uninfected individuals, indicating enhanced ROS-mediated LPO. HIV-1-induced ROS likely contribute to endothelium dysfunction through direct effects on the endothelium and/or indirectly through monocytes and macrophages contacting the vessel wall.

Elevated ROS in HIV-1 infection could play a role in various signaling pathways, among which are the mitogen-activated protein kinases (MAPKs). MAPKs are serine and threonine protein kinases, which have three major classes, including extracellular signal-regulated kinase 1 and 2 (ERK1/2) and BMK1, c-Jun N-terminal protein kinases (JNKs) and p38. ROS may mediate activation of MAPKs in a variety of cells, leading to changes in gene expression (Blenis, 1993), downregulation of eNOS, and alteration of other gene expression involved in the endothelium dysfunction. Taken together, these data indicate that oxidative stress activating MAPKs, may be one of the major mechanisms in HIV-1-induced endothelium dysfunction.

HIV-1 infected patients have low circulating levels of the antioxidant vitamin C, cysteine, and GSH, a situation that can lead to increased oxidative stress. Serum GSH levels and GSH peroxidase activity are decreased in HIV-1 patients, while the LPO product MDA, DNA fragmentation in lymphocytes, and total hydroperoxides are increased. These observations have important implications for therapeutic approaches. Clinical studies showed that selenium, and β carotene supplementation increased serum GSH levels. Dual vitamin C and E supplementation reduced plasma LPO and oxidative stress in HIV-1 patients. Supplementation with α-tocopherol or selenium also decreased plasma viral load and improved T-cell numbers and viability (Suresh et al, 2009; Stehbens, 2004).

These clinical findings suggest that vascular endothelial cells are exposed to ROS in the form of LPO products, pro-inflammatory cytokines, activated monocytes and phagocytes of the immune system.

## 5. Oxidative stress associated with antiretroviral therapy

Nearly 25 antiretroviral drugs have been licensed for the treatment of HIV-1 infected individuals and are divided mechanistically into five classes (reviewed by Estrada & Portilla, 2011): (1) nucleoside reverse transcriptase inhibitor (NRTI), including abacavir (ABC), didanosine (ddI), stavudine (d4T), lamivudine (3TC), tenofovir (TDF), zidovudine (AZT), and emtricitabine (FTC); (2) non-nucleoside reverse transcriptase inhibitor (NNRTI), including nevirapine (NVP), efavirenz (EFV), and etravirine (ETR); (3) protease inhibitor (PI) including atazanavir (ATV), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), darunavir (DRV), fosamprenavir (FPV), and amprenavir (APV); (4) fusion inhibitor, entry inhibitor (chemokine receptor CCR5 inhibitor) including enfuvirtide and maraviroc (MVC); and (5) integrase inhibitor, including raltegravir (RAL).

The HAART for management of HIV-1 infection that includes an association of the two NRTIs plus NNRTI and/or PI has been effective to suppress HIV-1 replication.

In addition to HIV-1 proteins, the HAART has been related with endothelium dysfunction. Experimental evidence shows that NRTIs are associated with endothelial cell toxicity. NRTIs induce oxidative stress, particularly mitochondrial ROS and seem to play an important role in cell culture and animal models of endothelial cell toxicity. However, clinical evidence for NRTIs-induced vascular/endothelium toxicity is indirect and difficult to define because NRTIs are not prescribed as monotherapy, and cardiovascular effects are often attributed to other components of HAART, such as PIs.

NNRTIs show, in general, the best lipid profile of all anti –HIV-1 drugs because they are associated with an increase in HDL cholesterol and a significant reduction in cholesterol total/HDL ratio. NNRTIs have been associated with a lower risk of myocardial infarction (Worm et al., 2010) that could hypothetically be associated with this good lipid profile. As regard NVP, the mechanism of HDL elevation may be an increase in the production of apolipoprotein-A1 (Franssen et al., 2009).

Among the PIs, lopinavir/ritonavir (LVP/r), DRV/r and ATV alone or with RTV (ATV/r) are the most extensively used PIs at present. PIs-associated dyslipidemia is a frequent class-related event and can limit their use especially in patients with preexisting increase cardiovascular risk. A meta-analysis of major clinical trials performed in 2009 (Hill et al., 2009) showed that patients randomized to LPV/r or FPV/r presented greater elevations of total cholesterol and triglycerides than those assigned to SQV/r, ATV/r, or DRV/r, without differences in low density lipoprotein cholesterol (LDL) or HDL.

The integrase inhibitor RAL is the first drug in this class and shows a remarkable lack of relevant adverse effects (Emery et al., 2010) and patients treated with RAL presented a significantly low frequency of dyslipidemia (Martinez et al., 2010).

Trials with HIV-1 patients treated with chemokine receptor-5 antagonist MVC have shown that it has a very favorable safety profile. MCV was associated with non-significant changes in total cholesterol, LDL, HDL and triglycerides (Cooper et al., 2010).

The LDL receptor (LDLR) plays a critical role in the regulation of plasma LDL levels (Brown & Goldstein, 1986). By controlling LDL catabolism, the number of hepatic LDLR directly governs the plasma LDL concentrations. The expression of LDLR is under metabolic, hormonal, and genetic control. Growth hormone (GH), insulin, estrogen, and dehydroepiandrosterone (DHEA) may stimulate LDLR expression and reduce plasma LDL cholesterol levels (Wade et al., 1989; Pascale et al., 1995; Rudling et al., 1996). As important hormonal modifications occur in HIV-1 infected patients with lipodystrophy, particularly insulin and DHEA changes, the LDLR expression was evaluated in HIV-1 infected patients with or without lipodystrophy. The results revealed that HIV-1-lipodystrophy is associated with a low expression of LDLR and this decreased expression seems independent of DHEA or insulin secretion (Petit et al., 2002). These authors suggested that the decreased expression of the LDLR may be explained by a direct effect of the PIs (Rayes et al., 1996). Other hypothesis is that PIs lead to dyslipidemia by inhibition of LDLR-related protein (LRP), which has homology to the catalytic site of HIV-1 protease, to which all PIs bind (Carr et al., 1998).

The HIV-1 infected patients with lipodystrophy have also an impaired metabolism of DHEA and insulin, all known to regulate LDLR (Meyer et al., 1998; Walli et al., 1998; Christeff et al., 1999; Behrens et al., 1999;). In addition, HIV-1 PIs also can modulate the function of certain LDLR family members. Tran et al., (2003) demonstrated that among six different HIV-1 PIs

evaluated, NFV, specifically, decreased mRNA and protein levels of the LDLR and LRP, which, in turn, decreased the functional activity of these two receptors. One study showed that exposure of IDV or NFV, combined with AZT and EFV, increased ICAM-1 gene expression and that concomitant exposure to TNF-α further increased ICAM-1 gene expression, VCAM-1, and endothelial-leukocyte adhesion molecule cell surface protein levels (Mondal et al., 2004).

The Figure 1 shows the production of NO induced by HIV-1 and its viral proteins (mainly gp120 and Tat proteins), and by PIs in promoting beneficial and deleterious effects in the host cells. The NO is synthesized via MAPK signaling pathway when the macrophage is activated by pro-inflammatory and inflammatory cytokines as result of the HIV-1 infection. Despite the protective effect of NO in the host defense against this pathogen, NO has been associated with a harmful effect in many systems.

The Figure 2 summarizes some of the effects of ROS and RNS that are induced by HIV-1 proteins and PIs.

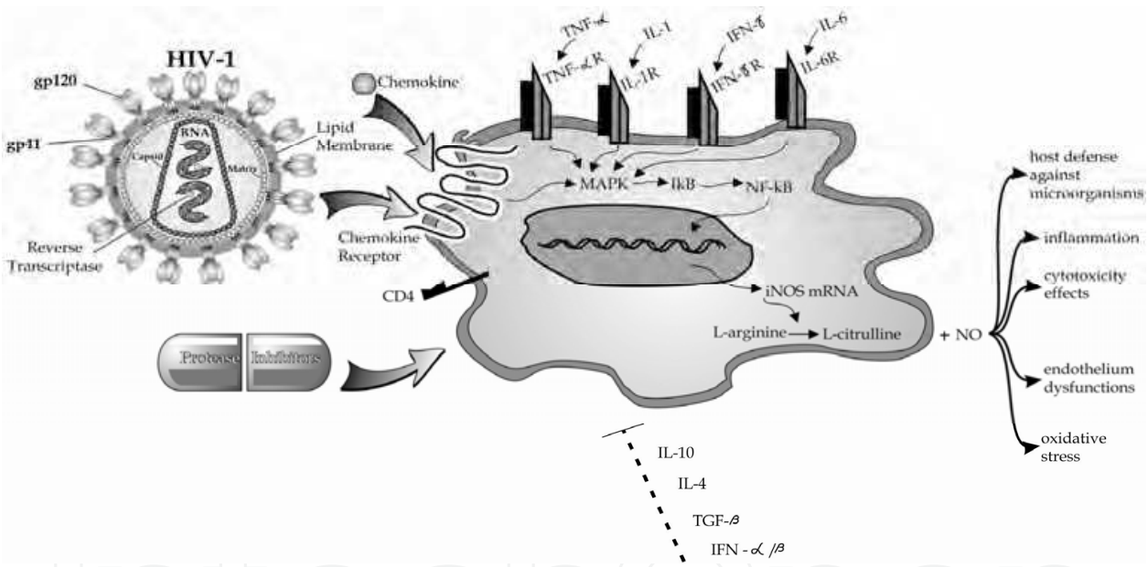


Fig. 1. Mechanisms of nitric oxide (NO) production induced by human immunodeficiency virus type 1 (HIV-1) and the viral proteins (mainly gp120 and Tat proteins), and by protease inhibitors (PIs) in promoting beneficial and deleterious effects in the host cells. TNF-α: tumor necrosis factor alpha;

TNF-αR: tumor necrosis factor alpha receptor; IL-1: interleukin 1; IL-1R: interleukin 1 receptor; IFN-γ: interferon gamma; IFN-γR: interferon gamma receptor; IL-6: interleukin 6; IL-6R: interleukin 6 receptor; IL-10: interleukin 10; IL-4: interleukin 4; TGF-β: transforming growth factor beta; IFN-α/β: interferon alpha and interferon beta; MAPK: mitogen-activated protein kinase; IkB: kinase inhibitor nuclear factor-kB; transcriptional nuclear factor kappa

beta (NF-kB); iNOS: inducible nitric oxide synthase; mRNA: messenger RNA; NO: nitric oxide.

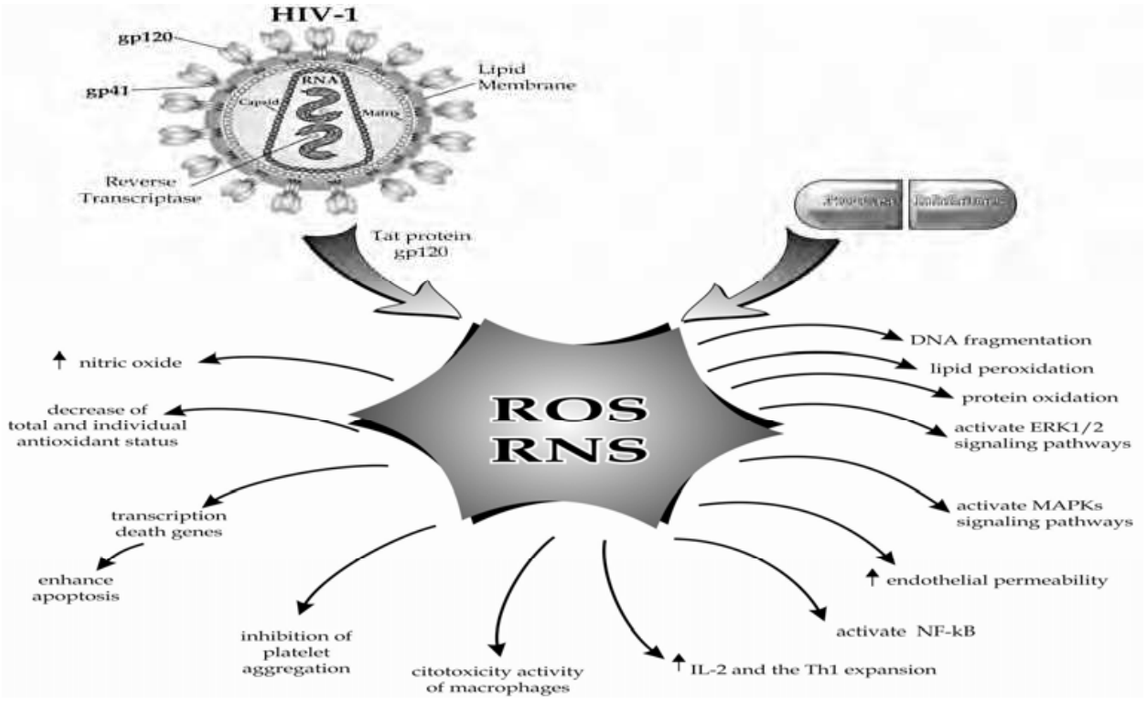


Fig. 2. Beneficial and deleterious effects of the endogenous reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are induced by both human immunodeficiency virus type 1 (HIV-1) proteins and the antiretroviral therapy with protease inhibitors (PIs). ROS and RNS that are accumulated by the imbalance of oxidants and antioxidants molecules exert effects on DNA, lipids, proteins, signaling pathways, immune system cells, neuronal tissue, and endothelial functions. ERK1/2: extracellular signal-regulated kinase 1 and 2; MAPKs: mitogen-activated protein kinases; transcriptional nuclear factor kappa beta (NF-kB).

## 6. Molecular mechanisms of HIV-1 PI-induced endothelium dysfunction

It is well-known that the endothelium acts as the first-line defense mechanism against the development of vascular injury. It exerts is protective action through modulation of vascular tone, vascular structure, and the interaction of blood components. Endothelial dysfunction may contribute to the systemic manifestations of many diseases, including atherosclerosis. Several reviews have been focused on metabolic disorders such as systemic insulin resistance, dyslipidemia, and peripheral lipodystrophy associated with endothelial dysfunction (Shankar & Dube, 2003; Koutkin & Grinspoon, 2004).

The molecular mechanisms of PIs toxicity in endothelial cells have been described in greatly detail. The effect of PIs on endothelium-depending vasorelaxation was first suggested by the significant reduction of flow-mediated vasodilatation of the brachial artery in HIV-1 infected patients receiving PIs as compared with the patients without PIs treatment (Stein et al., 2001). Other study showed that RTV, APV, or SQV individually caused a significant

reduction in endothelium-dependent vasorelaxation of porcine coronary arteries (Conklin et al., 2004). The expression of eNOS was significantly decreased in porcine coronary arteries treated by RTV, SQV, and APV. In parallel, RTV also caused a significant reduction of eNOS messenger RNA (mRNA) and protein levels in cultured human coronary artery endothelial cells (Fu et al., 2005).

PIs produces serious mitochondrial disturbances as evidenced by reduced cellular respiration and ATP production, decrease mitochondrial membrane potential, increase mitochondrial production of ROS, and enhanced mitochondrial DNA (mtDNA) damage. PIs also increase endothelial cell permeability and leukocyte adhesion in cell culture models. PIs contribute to cardiovascular risk by dysregulating fat cell homeostasis that may explain the high incidence of lipodystrophy and hyperlipidemia in HIV-1 patients. PIs prevent the differentiation of preadipocytes by decreasing matrix metalloproteinase expression, inhibiting adiponectin secretion, and inhibiting triglyceride and very low-density lipoprotein (VLDL) cholesterol clearance and catabolism (Wang et al., 2007). Evidences point to adipocytes as a complex and active endocrine tissue whose secretory products, including adiponectin, play an important role in the regulation of human metabolic alterations and vascular biology (Hamdy, 2005). Adiponectin accounts for approximately 0.01% of total plasma protein and has been shown to be related to lipodystrophy, metabolic alterations, and HIV-1 PIs use. Unlike other adipocyte products, adiponectin correlates with decreased free fatty acid blood concentrations and reduced body mass index. Adiponectin provides protection from vascular diseases by inhibiting local inflammatory signals, preventing preatherogenic plaque formation, and impeding arterial wall thickening (Schondorf et al., 2005). However, HIV-1 PIs such as RTV selectively decreased expression of adiponectin (Kim et al., 2006) suggesting that hypoadiponectinemia is partially responsible for the metabolic disorders induced by HIV-1 PIs, and adiponectin or its agonists might be used for the treatment of these disorders (Xu et al., 2004) .

HIV-1 PIs may also activate different types of MAPKs in different cell types or different culture conditions (Wang et al., 2007), leading to changes in gene expression in the same manner of the HIV-1 induced ROS.

Some studies have showed high oxidative stress among the effects of HAART. Mandas et al. (2009) assessed serum oxidant and antioxidant levels in HIV-1 infected population treated with HAART and compared them with those untreated HIV-1 seropositive and HIV-1 seronegative individuals. Serum oxidant levels were significantly higher in the HIV-1 treated group as compared to untreated and control groups. In addition, a decrease of serum total antioxidant status was observed in HIV-1 treated individuals. An important result obtained is that patients who rigorously followed antiretroviral therapy have significantly higher oxidative status than those who have poor HAART adherence. These results indicate that HAART may affect oxidative stress in HIV-1 infected patients and also suggested that antiretroviral therapy may exert a synergic effect with HIV-1 in the oxidative stress induction.

Another study (Gil et al., 2010) evaluated the effect of two HAART combinations on redox indicators and on progression markers of disease. A cohort of 84 healthy and 84 HIV-1 seropositive subjects was followed for six months. Fifty-six HIV-1 seropositive subjects were distributed in group I (AZT, 3TC, IND) and group II (d4T, 3TC, NEV) according to drug combination. Biomarkers of oxidative stress were evaluated including peroxidation

potential (PP), MDA, total hydroperoxides (HPO), AOPP and, percent of DNA fragmentation (% FDNA). There were also evaluated biomarkers of antioxidant status, including catalase, SOD and GSH at baseline and six months after HAART started. In this study, the concentration of antioxidants was low at baseline, and LPO index and DNA fragmentation were increased. After HAART had been started, catalase values for both groups receiving treatment showed no significant difference. For group II, all other parameters of oxidative stress were significantly higher than those for group I and the HIV-1 positive not treated, except for GSH values in group II which was lower than group I values. These data suggest poor prognostic for group II. The findings suggest that increased oxidative stress occurs additionally to persistent redox imbalance associated to HIV-1 infection during apparently successfully HAART.

HAART may increase chemically reactive species in circulation, possibly by producing more oxidized metabolites derived from the interaction between ROS and infected-cell biomolecules. This is supported by several biochemical mechanisms, such as mitochondrial interference, following treatment with HAART-NRTI and activation of the P450 hepatic system by HAART, when comprising PIs (La Asuncion et al., 1998; Kumar et al., 1999; Hulgan et al., 2003; Lewis, 2003; Cossarizza & Moyle, 2004; Day & Lewis, 2004).

## 7. Oxidative stress in HIV-1 infection associated with neurological disorders

The mechanisms by which HIV-1 first enters in the central nervous system (CNS) remain obscure. However, loss of blood-brain-barrier (BBB) integrity may be an important part of some of the tissue damage that accompanies HIV-1 infection of the brain, and may facilitate viral entry into the CNS. The active replication of HIV-1 into macrophages and microglia represents a reservoir for the virus and an important step for the neuropathogenesis of HIV-1 infection. This process leading to the production of inflammatory products and, in turn, to the production of an excess formation of free radical species, is involved in the subsequent increased permeability of the BBB and has been suggested to play a key role in the neuropathogenesis of HIV-1 infection. The combination of BBB damage and elevated plasma viral load is associated with neurocognitive impairment and an increased risk for development of HIV-1-induced dementia (HIVD). In addition, oxidative stress has been demonstrated in the brain and cerebrospinal fluid (CSF) from HIV-1-infected individuals and it is proposed to be a key event in the pathophysiology of HIVD.

One of the neurotoxins that is suggested to be involved in neuronal damage is NO. NO is a nitrogen free radical generates in many tissues, including the CNS, via bioconversion of L-arginine into L-citrulline by nNOS (Lamas et al., 1998). It can be released constitutively by neurons in response to many neurochemical stimuli, including excitatory neurotransmission and changes of $Ca^{2+}$ influx (Moncada et al., 1991). NO release has been induced *in vitro* from glial cells following the addition of inflammatory cytokines and soluble antigens such as the HIV-1 coating gp120 glycoprotein (Dawson et al., 1993; Mollace & Nistico, 1995). Pro-inflammatory cytokines including IL-1, TNF-α, and IFN-γ which are released in HIV-1 infected brain tissue have been shown to upregulate the iNOS. To modulate this response, the NO formation is downregulated by the cytokines tissue grown factor beta (TGF-β), and IFN alpha/beta (IFN-α/β), according to Hua et al., (1998).

Evidences show that although the direct neurotoxic effects of NO are modest, they are greatly enhanced by reacting with superoxide anion to form peroxynitrite. Superoxide anion

is produced by myeloid-monocytic cell lines following HIV-1 infection and the production of this molecule results in subsequent changes in the antioxidant status of these cells because SOD, a superoxide anion scavenger, is generated. Neurofilament, a protein that provides structural stability to neurons, is one of the target proteins of peroxynitrite and the resulting nitration results in disrupted neurofilament assembly and thus neuronal damage (Coyle & Puttfarcken, 1993).

Neurotoxic levels of ROS and RNS are especially produced by the macrophages recruited to the CNS as well as by astrocytes and glial cells activated following different stimuli such as cytokine, endotoxin, and soluble antigens in the CSN.

*In vitro* studies show that gp120 and Tat HIV-1 proteins can be directly toxic to human endothelial cells, compromises BBB integrity by reducing tight junction (occludin) protein expression and enhances monocytes migration across BBB. Protein oxidation was increased in the CSF of HIV-1 patients with mild and severe dementia compared to non-dement HIV-1 patients. Nitrated tyrosine residues, evidence of peroxynitrite reaction with proteins, are increased in brain of HIVD patients. Activation of cytokine receptors and oxidative stress can induce the production of ceramide from membrane sphingomyelin, and recent findings suggest that ceramide is an important mediator of apoptosis. The HIV-1 Tat protein can also induce increase of ceramide and sphingomyelin in culture neurons. Tat can be transported efficiently across the intact BBB. In HIV-1 infected astrocytes, the regulatory gene *tat* is overexpressed, and mRNA levels for Tat protein are elevated in brain extracts from individuals with HIVD. The Tat sequences from brains of patients with HAD are mutated with glutamate substitutions in the second exon, which may decrease its ability to be taken up by cells, thus increasing its extracellular concentrations and possibly neurotoxicity effects in the cell. Brain regions particularly susceptible to Tat toxicity are striatum, hippocampal dentate gyrus, and the CA3 region of the hippocampus.

Tat has been hypothesized by many studies as a potential contributor to HIVD by many mechanisms (reviewed by Pocernich et al., 2005). Tat protein released by astrocytes produces trimming of neuritis, mitochondrial dysfunction, and cell death in neurons. Tat-induced neurotoxicity is though to be mediated through excitotoxic mechanisms involving calcium. Tat can also induce markers of oxidative stress such as protein and LPO in synaptosomal membranes and neuronal cell cultures. To neutralize the oxidative stress, the GSH protects neurons against ROS directly and indirectly, and binds LPO products. GSH is the major cellular thiol participating in the maintenance of cellular redox status of the neuron and neuronal mitochondria. The biosynthesis of GSH may be compromised by Tat protein. It was hypothesized that the chronic inflammation of CSN by HIV-1, the activation of microglia, and increased lipid and protein oxidation, all observed in HIV-1 infected patients, can lead to the decrease of GSH serum levels and potentially HIVD. Low serum level of GSH is associated with poor survival in HIV-1-infected patients, while administration of GSH to HV-1-infected patients decreases mortality.

The production of superoxide anions by HIV-1 infected cells is counteracted by SOD, which, in turn, generates hydrogen peroxide ($H_2O_2$). Under basal conditions this is scavenged by catalase. To date, clear evidence exists that catalase activity is modified in brain tissue of AIDS patients. However, it has recently been reported that catalase is diminished in $CD8^+$ T lymphocytes from HIV-1 positive individuals, suggesting the $H_2O_2$ scavenger activity might be decreased during HIV-1 infection (Yano et al., 1998).

## 8. Antioxidant status in HIV-1 infection

Although the concentration of plasma antioxidant components can be measured individually, these measurements may be time- and cost-consuming and labor intensive. In addition, it may not accurately reflect the total antioxidant status (Wayner et al., 1987). Total antioxidant capacity considers the cumulative effect of all oxidants present in blood and any fluids (Nagy et al., 2006) and it could be evaluated by several assays including total peroxyl radical trapping antioxidant parameter (TRAP), total antioxidant capacity (TAC), ferric reducing ability (FRAP), and their variations.

It has been previously shown that the HIV-1 infected individuals are oxidative stressed and have significantly lower antioxidant concentrations than HIV-1 seronegative individuals.

There is experimental evidence that different metabolic events that occur as consequence of HIV-1 infection directly influence the consumption of antioxidant components thus contributing to the increase of oxidative stress. Studies have found impaired antioxidant defense in HIV-1 infected patients and the antioxidant depletion indicates a decrease in immune function. Cells of immune system generally require a higher antioxidant concentration than other cells to retain redox balance, and preserve integrity and function (De La Fuente et al., 2002).

There are numerous studies reporting GSH deficiency in HIV-1 infection. The concentration of GSH is low in plasma, lung epithelial lining fluid, and peripheral blood mononuclear cells of HIV-1 infected individuals (Buhl et al., 1989; Roederer et al., 1993). Studies *in vitro* have shown that low GSH levels impair T cell function and also promote HIV-1 expression, suggesting a link between GSH deficiency and progression of HIV-1 disease (Kalebic et al., 1991; Roederer et al., 1993). Poor survival rates of HIV-1 seropositive individuals with low GSH levels and improved survival when GSH was replenished were also reported (Herzenberg et al., 1997). Taken together, these data can proposed that a persistent oxidative stress leads an accelerated rate of consumption of GSH that is not matched by an equal in the rate of synthesis of the tripeptide.

Gil et al. (2003) showed both a reduction of GSH levels and an increased in MDA and total hydroperoxides levels were detected in the plasma of HIV-1 seropositive individuals. These patients also showed an increase of DNA fragmentation in lymphocytes, reduction of glutathione peroxidase, and an increase in SOD activity in erythrocytes. There are several studies of disturbed GSH metabolism in HIV-1 infected patients. Arkrust et al. (2003) showed that, during HAART, the decrease in virus load and the increase in $CD4^+$ T cell count are accompanied by both an improvement in the abnormal GSH-redox status and an increase in the subnormal levels of antioxidant vitamins. They have shown that HIV-1 infected patients are characterized by a decrease in both reduced GSH and vitamin C, the two most important hydrophilic antioxidants.

HIV-1 infection results in considerably reduced α-tocopherol concentrations and very low plasma zinc and selenium levels. Zinc and copper ions inhibit intracellular HIV-1 replication (Sprietsma, 1997). The precise mechanism by which the antioxidant effects of zinc is accomplished stems from its involvement in SOD and other enzymatic process. In humans, marked zinc deficiency strongly compromises the immune function and often enhances vulnerability to fatal opportunistic infections. It decreases $CD4^+$ T helper cell function, $CD8^+$ T cell cytotoxic activity, serum thymulin activity, and the interleukin-2 (IL-2) production by peripheral blood mononuclear cells. It also reduces the natural killer

cells lytic activity, DNA repair, the antibodies formation, and macrophage and neuthrophil function. In experimental and human models, the zinc deficiency caused an imbalance between Th1 and Th2 functions resulting in decreased production of IFN-γ. These specific effects on T cell proliferation and function are not duplicated by other micronutrients (Stehbens, 2004).

Selenium deficiency diminishes cell-mediated immunity and depresses B-cell function, and it is associated with the occurrence, virulence, and disease progression to overt AIDS (Stehbens, 2004). Apoptosis of the cells is fundamental to progression of the disease that correlates with the decrease in plasma zinc, selenium, and vitamin E (Farvier et al., 1994).

Many antioxidants have been tried for AIDS therapy including selenium, vitamin C, vitamin E, lipoic acid, β carotene, whey proteins, and the epigallocatechin gallate (EGCG), the major component of green tee.

However, there are conflicting reports in the values of antioxidant vitamin E and vitamin C and SOD enzyme activity among HIV-1 infected patients in various stages in the literature (Allard et al., 1998; Stambullian et al., 2007). Suresh et al. (2009) showed that vitamin E, vitamin C, SOD, and TAC levels are decreased in HIV-1 patients, and the depletion was pronounced in HIV-1 symptomatic compared to HIV-1 asymptomatic individuals, in contrast to previous studies where were no significant differences in antioxidant vitamins in both groups (Allard et al., 1998).

McDermid et al. (2002) investigated the relation between dietary antioxidant intake and oxidative stress in clinically stable HIV-1 positive and HIV-1 negative adults. The results suggested dietary selenium intake was strongly and inversely associated with plasma MDA, but dietary antioxidant intakes were not related to peripheral blood mononuclear cell GSH concentration.

Total antioxidant status has been reduced in HIV-1 infected patients, probably due depletion of antioxidant molecules when they are consumed in the process of protecting cells against ROS induced oxidative damage in a magnitude that is related to advancement of the disease to AIDS (Ogunro et al., 2005).

Endothelial dysfunction induced by HIV-1 PIs may possible be reversed by antioxidants, including ginsenosides, selenium, curcumin (Chai et al., 2005a; Chai et al., 2005b), and resveratrol (Touzet & Philips, 2010). Therefore, it has been proposed by some researches that the oxidative stress and antioxidant status of HIV-1 seropositive patients could be monitored periodically during the disease progression. The possibility of counteracting oxidative stress by a pool of proper antioxidant plus an appropriate diet, mainly in patients whose blood antioxidant deficiencies can be easily rebalanced may have real health benefit and represent a promising way of inhibiting the progression of disease.

A new class of non-peptidic macrocyclic (MnII) complexes that possesses SOD enzymatic activity has been synthesized, which has the same activity as native SOD but can significantly cross the BBB (Salvemini et al., 1999). A SOD mimetic complex has been shown to significantly protect against the apoptotic cell death that occurs in astroglia that was incubated with supernatants of HIV-1 infected human macrophages. This effect was accompanied by a reduction of MDA concentration in astroglial cells and by a reduction of nitrotyrosine staining in these cells, showing that the effect of this mimetic complex occurred via reduction of ROS formation, and in turn, could reduce the neurodegenerative processes that occur in neuroAIDS (Mollace et al., 2001).

Many clinical trials on HIV-1 dementia have centered on drugs that block receptors or are antagonists to the neurotoxic chemokines and cytokines released from activated microglia, macrophages, and astrocytes. These drugs, including nimodipine (L-type calcium channel antagonist), peptide T (possible chemokine receptor blocker), selegiline and deprenyl (monoamine oxidase-B inhibitors), lexipafant (platelet-activating factor antagonist), and CPI-1189 (TNF antagonist), indirectly act as antioxidants by blocking the downstream effects of these neurotoxic agents that usually result in an increase of ROS, RNS, and neuronal death (Turchan et al., 2003).

The importance of micronutrients in the prevention and treatment of childhood infections is well known, and evidence is emerging that micronutrient interventions may also affect HIV-1 transmission and progression. To clarify this issue, Friis (2006) reviewed evidences on the role of micronutrient supplementation in HIV-1 transmission and progression. The author concluded that interventions to improve micronutrient intake and status could contribute to a reduction in the magnitude and impact of the global HIV-1 epidemic. However, more research is needed before specific recommendations can be made. Fawzi et al (2005) underscored that poor nutrition and HIV-1 related adverse health outcomes contribute to a vicious cycle that may be slowed down by using nutritional interventions, including vitamins and minerals. Among children, periodic supplementation with vitamin A starting at six months of age has been shown to be beneficial in reducing mortality and morbidity among both HIV-1-infected and uninfected children. Limited data exist on the role of other nutrient supplements among children. Among HIV-1 infected adults, the safety and the efficacy of vitamin A supplements need further study, although adequate dietary intake of this essential nutrient is recommended. Multivitamin supplements were efficacious in reducing adverse pregnancy outcomes and early childhood infections, and is currently provided to HIV-1 infected pregnant women in many programs. The efficacy of such supplements among HIV-1 negative pregnant women needs further study. Daily multivitamin supplements were found to reduce HIV-1 disease progression among men and women and could be provided to adults in early stages of HIV-1 disease to prolong the time before antiretroviral therapy.

In order to assess whether micronutrient supplements are effective and safe in reducing mortality and morbidity in adults and children with HIV-1 infection, 30 randomized controlled trials were selected that compared the effects of micronutrient supplements (vitamins, trace elements, and combinations of these) with other supplements, placebo or no treatment on mortality, morbidity, pregnancy outcomes, immunologic indicators, and anthropometric measures in HIV-1 infected adults and children (Irlam et al, 2005, 2010). Any adverse effects of supplementation were recorded in 30 trials involving 22,120 participants: 20 trials of single supplements (vitamin A, vitamin D, zinc, selenium) and 10 of multiple micronutrients. Eight trials were undertaken in child populations. The results of this meta-analysis showed that multiple micronutrient supplements reduced morbidity and mortality in HIV-1 infected pregnant women and their offspring and also improved early child growth in one large randomized controlled trial in Africa. Additional research is needed to determine if these are generalisable findings. Vitamin A supplementation is beneficial and safe in HIV-1 infected children, but further evidence is needed to establish if supplementation confers similar benefits in HIV-1 infected adults. Zinc is safe in HIV-1 infected adults and children. It may have similar benefits in HIV-1 infected children and adults, and uninfected children with diarrhea, as it does in HIV-1 uninfected children. Further trials of single supplements (vitamin

D, zinc, and selenium) are required to build the evidence base. The long-term clinical benefits, adverse effects, and optimal formulation of multiple micronutrient supplements require further investigation in individuals with diverse disease status.

The exogenous supply of antioxidants using novel and more-specific molecules that scavenge free radical might allow further advances in understanding the processes that underlie the pathogenesis of HIV-1 infection and thus might represent the basis for novel and potentially efficient strategies in the complementary treatment of neurological, endothelium, and cardiovascular diseases associated with the HIV-1 infection.

## 9. Conclusion

There is clear evidence that the gp120 and Tat HIV-1 proteins and antiretroviral drugs directly and indirectly induce oxidative stress. Damage-induced by oxidative stress in endothelial cells and neurons may be correlated with an increase in the risk of cardiovascular disease and dementia, respectively, in HIV-1 infected patients. Although differences may exists to the relative contribution and mechanisms of toxicity, the preponderance of clinical and experimental data suggest roles for both of these factors in the context of HIV-1 infection. In assessing cardiovascular risk, it is important to take into account potential contributions from both infection and therapy. To various degrees, multiple HIV-1 viral proteins and antiretroviral drugs activate cell signaling cascades, induce oxidative stress, disturb mitochondrial function, alter gene expression, and impair lipid metabolism. These changes occur in endothelial cells, in vascular muscle cells, macrophages, adipocytes, and in neuronal cells.

The main changes that have been reported by *in vivo* and *in vitro* studies are the increase of the LPO, protein oxidation, and NO metabolites, decrease in the individual antioxidants defenses such as vitamin C, vitamin E, GSH, catalase, selenium, and zinc. In addition, the total status antioxidant is also impaired in HIV-1 infected individuals. NO cannot be rigidly classified as an anti-inflammatory or pro-inflammatory molecule, but it can be considered a true inflammatory mediator. It has been also reported that oxidative stress in HIV-1 infected individuals is associated with increase of DNA fragmentation in lymphocytes, reduction of glutathione peroxidase, and an increase in SOD activity in erythrocytes.

The better knowledge of the ways in which HIV-1 proteins and antiretroviral drugs interact with each other and with the host cells, mainly the endothelial, the neuronal, and immune system cells, may contribute to discover new approaches to be associated with the antiretroviral therapies in order to prevent cardiovascular diseases and neurological disorders in HIV-1 infected individuals.

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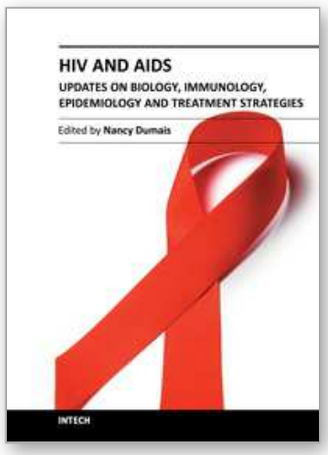


**HIV and AIDS - Updates on Biology, Immunology, Epidemiology and Treatment Strategies**

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The continuing AIDS pandemic reminds us that despite the unrelenting quest for knowledge since the early 1980s, we have much to learn about HIV and AIDS. This terrible syndrome represents one of the greatest challenges for science and medicine. The purpose of this book is to aid clinicians, provide a source of inspiration for researchers, and serve as a guide for graduate students in their continued search for a cure of HIV. The first part of this book, â€œFrom the laboratory to the clinic,â€ and the second part, â€œFrom the clinic to the patients,â€ represent the unique but intertwined mission of this work: to provide basic and clinical knowledge on HIV/AIDS.

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