Review

HIV-dementia, Tat-induced oxidative stress, and antioxidant therapeutic considerations

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Abstract

Oxidative stress is thought to play a role in the onset of dementia. HIV-dementia has recently been demonstrated to be associated with oxidative stress as indexed by increased protein and lipid peroxidation in the brain and cerebrospinal fluid compared to HIV non-demented patients. The HIV protein Tat induces neurotoxicity, and, more recently, Tat was found to induce oxidative stress directly and indirectly. The role of Tat in HIV-dementia and possible therapeutic strategies involving endogenous and exogenous antioxidants are discussed.

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Topic: Infectious diseases
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Abbreviations: HIV, human immunodeficiency virus; HIVD, human immunodeficiency virus-dementia; AD, Alzheimer’s disease; CSF, cerebrospinal fluid; ROS, reactive oxygen species; HNE, 4-hydroxy-2-nonenal; GSH, glutathione; CNS, central nervous system; NAC, N-acetylcysteine; TRX, thioredoxin; EGCG, epigallocatechin gallate; PKC, protein kinase C; PI, phosphatidylinositol; PLC, phospholipase C; DAG, diacylglycerol; IP\textsubscript{3}, inositol triphosphate; ER, endoplasmic reticulum; LRP, low-density lipoprotein; TNF, tumor necrosis factor; IL, interleukin; INOS, inducible nitric oxide synthase; NO, nitric oxide; QUIN, quinolinic acid; ApoE, apolipoprotein E; D609, tricyclodecan-9-yl-xanthogenate

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1. Introduction

It is estimated that one-third of adults infected with human immunodeficiency virus (HIV-1) develop dementia [70]. HIV-1 dementia (HIVD) is now the leading cause of dementia in people younger than 60 years of age [105]. Oxidative stress is thought to play a role in the onset of dementia associated with Alzheimer’s disease (AD) [21,22,25,103]. Recently, we reported that oxidative stress has been demonstrated in the HIV brain and cerebrospinal fluid (CSF) [159]. These observations have important implications for therapeutic approaches for HIV-dementia.

2. Oxidative stress

Oxidative stress can be defined as the modification and accumulation of biological molecules altered by various kinds of reactive oxygen species (ROS). Oxidative stress is also defined as an imbalance between the antioxidant and the pro-oxidant systems, with the shift towards the pro-oxidant system. ROS are highly reactive, toxic oxygen moieties, such as hydroxyl radical, peroxy radical, superoxide anion, hydrogen peroxide, and peroxynitrite. The half-life of ROS species varies from nanoseconds for the hydroxyl radical to seconds for nitric oxide and peroxyl radicals. Because of the differences in half-lives, the ROS reactivity differs from the aqueous environment in which they were formed (hydroxyl radical) to reacting deep within the membrane (H$_2$O$_2$).

Collectively, ROS can lead to oxidation of proteins and DNA, peroxidation of lipids, and ultimately cell death [24]. Protein carbonyl groups are used as a marker of protein oxidation [23]. 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 lipid peroxidation product 4-hydroxynonenal (HNE) to proteins [23,150]. Alterations in proteins can lead to aggregation, changes in secondary and tertiary structure, susceptibility to proteolysis, fragmentation, and loss of function. Lipid peroxidation produces large amounts of aldehydes, such as HNE, malondialdehyde, and acrolein, and leads to isoprostane formation [27]. HNE and acrolein contribute to membrane damage and cell death induced by a variety of oxidative insults [48], and through alterations of protein structure [153] are capable of inhibiting DNA, RNA, and protein synthesis, glycolysis, and degradation of enzymes.

3. Oxidative stress and HIV-dementia

Oxidative stress in HIV-dementia patients has been demonstrated in the brain and CSF [33,159]. Staining of HNE was found to be prominent in neurons, glial cells, and perivascular cells in brain slices of patients with HIV encephalitis and macaques with SIV encephalitis [159]. Protein oxidation was increased in the CSF of HIV-patients with mild and severe dementia compared to non-demented HIV-patients. Nitrated tyrosine residues, evidence of peroxynitrite reaction with proteins, is increased in HIVD brains [14]. 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 a form of programmed cell death (apoptosis). Recently, it was reported that levels of ceramide, sphingomyelin, and HNE are significantly increased in brain and CSF of HIVD patients, and the HIV protein Tat can induce increases of all three in cultured neurons [61]. Mass spectrometry studies of HNE, sphingomyelin, and ceramide were performed on autopsy tissue and CSF of HIV patients prior to the use of highly active antiretroviral therapy (HAART). HNE in the CSF of HIV patients with inactive HIVD (dementia status did not change in the last 6 months) slightly increased and greatly increased with active HIVD (a transition from a non-demented status to dementia within 6 months) compared to HIV patients with no dementia (HIV-ND) [136]. Sphingomyelins were elevated in mild HIVD cases compared to HIV-ND in the CSF and medial frontal gyrus, while moderate HIVD cases were elevated only in the CSF. Ceramide levels were elevated in mild HIVD cases compared to HIV-ND in the CSF and medial frontal gyrus, while moderate HIVD cases were elevated only in the CSF. Ceramide levels were elevated in mild HIVD cases compared to HIV-ND in the CSF and medial frontal gyrus. Sphingomyelin and ceramide can vary in length from 16 to 24 carbons. Different length sphingomyelins and ceramides were significant in different brain regions of HIVD patients (for review, see [136]).

4. HIV and antioxidants

To counteract these damaging radicals, antioxidant systems have evolved, including enzymes like glutathione peroxidase, glutathione reductase, glutathione transferase, superoxide dismutase, S-methyl transferase, and catalase. Protection against free radicals can also come from small, non-protein, cellular antioxidants, such as glutathione,
Antioxidant levels in HIV-infected patients are altered, a situation that can lead to increased oxidative stress. The tripeptide glutathione (γ-glutamate-cysteine-glutamate, GSH), present in millimolar concentrations in the brain, functions as an antioxidant and maintains sulfhydryl groups of proteins in the reduced form [126]. Glutathione protects neurons against reactive oxygen species directly and indirectly, and binds lipid peroxidation products such as HNE, thereby providing neuroprotection [41,127,126]. Glutathione levels are decreased in HIV patients (Table 1). Serum glutathione levels and glutathione peroxidase activity were significantly lower in HIV than in controls, while the lipid peroxidation product malondialdehyde, DNA fragmentation in lymphocytes, and total hydroperoxides were increased [56]. The concentrations of GSH and other sulfhydryl compounds are decreased in the blood, liver, and central nervous system (CNS) of HIV-infected patients [31,36], and low GSH is associated with poor survival in HIV-infected patients, while administration of GSH to HIV-infected patients decreases mortality [64].

N-Acetyl-L-cysteine (NAC) acts as an indirect precursor of glutathione by raising intracellular levels of cysteine, a precursor of glutathione [126,127]. NAC also has antioxidant properties of its own due to the sulfhydryl group. We demonstrated that NAC injected i.p. into rodents increases glutathione levels in the brain and protects brain against the damaging effects of hydroxyl radicals and the lipid peroxidation product acrolein [126,127]. In vitro, NAC inhibits viral replication in human monocyte-derived macrophages and lymphocytes [66,74,109]. The administration of NAC to HIV-infected patients has been shown to decrease mortality [64]. Recently, various NAC analogs including N-(N-acetyl-L-cysteinyl)-S-acetylcysteamine have been shown to increase glutathione and display anti-HIV activity making them possible therapeutic candidates for HIV infection [41,42,122,126,127].

Whey proteins have been shown to increase glutathione levels in humans, most likely by supplying the amino acid cysteine necessary for the synthesis of glutathione. HIV patients receiving a daily dose of whey proteins had a significant elevation of plasma glutathione levels after 2 weeks [107,108]. The exogenous supplement action of compounds that increase concentrations of brain glutathione such as gamma-glutamylcysteine ethylester [41,42,126,127], might allow further advances in understanding the processes underlying HIV-dementia and provide additional strategies in the treatment of HIV and HIV-dementia.

Thioredoxin (TRX) is a redox active thiol similar to glutathione. TRX reduces protein disulfides with the aid of TRX reductase and is a crucial antioxidant in the extracellular space where glutathione levels are limited [114]. Plasma levels of TRX are significantly elevated in HIV-infected individuals compared to healthy controls [113] and inversely correlates with decrease in intracellular GSH levels in T cells (Table 1). The data suggest that oxidative stress in HIV infection leads to decreases of intracellular GSH levels and increases of plasma TRX levels.

The major component of green tea, epigallocatechin gallate (EGCG), has been shown to have antiviral, antitumorigenic, anti-inflammatory, antioxidant, antibacterial, and antiproliferative effects [26,116]. Several mechanisms for the antiviral effects of EGCG on HIV-1 have been proposed. EGCG inhibits the HIV protein gp120 from binding to the host cell surface by binding the CD4 receptor on the cell surface [78]. Entry of the HIV virion is achieved by gp120 binding to the CD4 and chemokine receptors on the host cell surface [85]. EGCG also inhibits HIV-1 replication by blocking the activity of HIV-1 reverse transcriptase [49,115]. Lastly, EGCG induces virion destruction by deformation of phospholipids by binding to the surface of the viral envelope [168]. Polyphenols also have antioxidant capabilities [26]. HIV-positive patients who drank fruit and vegetable juices had increased lymphocyte proliferation, which could restore disturbances in T-cell homeostasis [165]. Moreover, polyphenols such as curcumin and ferulic acid can induce stress response protective genes, such as heat shock protein-32 (heme oxygenase-1) [29,30,140].

Many other antioxidants have been tried for AIDS therapy including selenium, vitamin C, vitamin E, lipoic acid, and β-carotene. Selenium levels in blood of HIV-infected subjects are decreased [123]. Selenium supplementation increases glutathione peroxidase activity [138] and inhibits TNF-α-induced HIV replication [67]. Recently, lower plasma selenium levels were significantly associated with an increased risk of mortality in pregnant women from Tanzania followed over a 5- to 7-year period [83]. Vitamin C suppresses the replication of HIV by reduction of reverse transcriptase activity [58], and vitamin E suppresses the activation of NF-κB [69]. Supplementation of vitamin E and vitamin C was found to reduce oxidative stress in HIV infection and produced a downward trend in HIV viral load in a blind HIV study [3]. HIV-infected patients supplemented with vitamins A, C, and E had significantly decreased levels of oxidized DNA bases and lipid peroxidation, and had increased activity of antioxidant enzymes superoxide dismutase and catalase.

Table 1

<table>
<thead>
<tr>
<th>Evidence</th>
<th>Body site/cell type</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>GSH and cysteine depletion</td>
<td>Plasma, blood, liver, CNS</td>
<td>[18,31,36,43,45,56,148,149]</td>
</tr>
<tr>
<td>GSH depletion/ GSSG elevation</td>
<td>Peripheral blood T cells</td>
<td>[8,94,147]</td>
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<tr>
<td>TRX depletion</td>
<td>Plasma</td>
<td>[102]</td>
</tr>
<tr>
<td>TRX elevation</td>
<td>Peripheral blood T cells, monocytes, plasma</td>
<td>[79,97,113]</td>
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<tr>
<td>Selenium decreased</td>
<td>Blood</td>
<td>[123]</td>
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Recently, Tanzanian women who received vitamins B, C, and E supplementation were less likely to have progression to advanced stages of HIV disease, had better preservation of CD4+ T-cell counts and lower viral loads, and had lower HIV-related morbidity and mortality rates than women who received placebo [52]. HIV-infected Thai adults who received multivitamins also had decreased mortality rates [72]. Fawzi and colleagues have previously shown that multivitamin use is associated with improved pregnancy outcomes, including reduced rates of low birth weight, prematurity, and fetal death in HIV women [50]. Vitamin A supplementation has reportedly been followed by increased rates of mother-to-child transmission of infection [51]. Supplementation of lipoic acid (thioctic acid), a sulfur containing antioxidant, in patients with HIV-associated cognitive impairment, did not improve cognitive function [1]. Several reports have shown that β-carotene supplementation has no significant effect in HIV-infected individuals [38,39]. The above results suggest that HIV-infected people can benefit from selected antioxidant treatment. In fact, a clinical trial using minocycline is being initiated in patients with HIVD, which has both antioxidant and anti-inflammatory effects (Sacktor, Johns Hopkins University, personal communication).

Many clinical trials on HIV-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 indirectly act as antioxidants by blocking the downstream effects of these neurotoxic agents that usually result in an increase in reactive oxygen species and neuronal death. HIV-dementia clinical trials to date include nimodipine (L-type calcium channel antagonist), peptide T (possible chemokine receptor blocker), seleagine (monoamine oxidase-B inhibitor), deprenyl (monoamine oxidase-B inhibitor), lexipafant (platelet-activating factor antagonist), memantine (NMDA antagonist), and CPI-1189 (TNF inhibitor). Most of these drugs displayed a trend for improvement (for review, see [160]).

Memantine is an NMDA receptor antagonist that blocks the receptor’s ion pore, but has a low affinity for the NMDA receptor, allowing it to disassociate readily from the channel [124]. Memantine is currently used to treat Alzheimer’s disease, another dementing disorder [20]. In vitro, HIV-1 proteins gp120 and Tat activate NMDA receptors, and this activation is blocked by memantine [90,120]. Transgenic mice expressing the HIV-1 protein gp120, also benefited from memantine treatment [156]. Recently, memantine has been demonstrated to attenuate hippocampal synaptic impairment in murine HIVE [5]. Memantine is currently in a phase II study for HIV-associated dementia.

HIV-infected individuals supplemented with deprenyl, a monoamine oxidase B inhibitor and putative anti-apoptotic agent, displayed significant improvement on tests of verbal memory compared with patients not taking deprenyl [1].

5. Tat

In patients with HIV-1 infection, significant neuronal loss and dysfunction occur even though, ironically, neurons are rarely infected [121]. In brain, microglia, macrophages, and astrocytes are the most commonly infected cell types [80,84,137]. Uninfected cell death can be induced by viral proteins released from nearby HIV-1-infected cells and host-derived toxins. Toxins released from HIV-1-infected proteins include Tat and gp120.

Tat is a nonstructural HIV protein of 86–101 amino acids that is formed from 2 exons. The first exon contributes to the first 72 amino acids and acts as a transacting nuclear regulatory protein essential for viral replication. Tat can be transported efficiently across the intact blood–brain barrier [141]. In HIV-infected astrocytes, the regulatory gene tat is overexpressed [117], and mRNA levels for Tat are elevated in brain extracts from individuals with HIV-1 dementia [68,164]. The Tat sequences from brains of patients with HIV-dementia are mutated with glutamate substitutions in the second exon [15], which may decrease its ability to be taken up by cells, thus increasing its extracellular concentrations and possibly neurotoxicity effects on the cell. Brain regions particularly susceptible to Tat toxicity, i.e., striatum [63], hippocampal dentate gyrus, and the CA3 region of the hippocampus [99], parallel those significantly affected in Alzheimer’s disease. Tat has been hypothesized by many as a potential contributor to HIV-dementia [59].

6. Tat-induced neurotoxicity

Tat is a mediator of neurotoxicity. Tat transactivates viral and cellular gene expression, is actively secreted into the extracellular environment mainly from astrocytes, microglia, and macrophages, and is taken up by neighboring uninfected cells such as neurons [32,46,81,135]. The HIV-1 protein Tat released from astrocytes reportedly produces trimmings of neurites, mitochondrial dysfunction, and cell death in neurons [33]. A single injection of full-length Tat(1–86), Tat(1–72), or the short basic domain of Tat(48–57) into the hippocampus or thalamus resulted in glial cell activation, influx of inflammatory cells, induction of inducible nitric oxide synthase, and neurotoxicity [73,125]. In vivo measurements of Tat are difficult because Tat antibodies have weak affinity and are poorly standardized from laboratory to laboratory [37]. HIV-Tat has been detected in sera of HIV-infected patients at the low nanomolar level [163,167], a value that may be underestimated since Tat can be trapped by heparan sulfate, which is widely expressed on cell surfaces [134,171]. Furthermore, this concentration would be higher near productively infected cells. In vitro systems test Tat toxicity between 100 and 500 nM concentration.

Tat-induced neurotoxicity is thought to be mediated through excitotoxic mechanisms involving calcium. Tat is
capable of depolarizing rat CA1 hippocampal neurons and human cortical neurons [95], increasing intracellular Ca$^{2+}$ [118], and inducing neuronal death [63,95,155]. The increases of cytosolic calcium are followed by mitochondrial calcium uptake, generation of ROS, activation of caspases, and eventually apoptosis [82]. Cleavage of phosphatidylinositol (PI) by phospholipase C (PLC) to produce diacylglycerol (DAG) and inositol triphosphate (IP$_3$) has been implicated as an important signaling pathway for Tat. In neurons, Tat activates phosphatidylinositol 3-kinase [110], increases levels of IP$_3$, releases calcium from IP$_3$-sensitive endoplasmic reticulum (ER) internal stores [60], and increases activity of the protein kinase C isoforms α, ε, and ζ [13], all precursors of oxidative stress. Tat-induced neurotoxicity is prevented by antagonists of phospholipase C and IP$_3$-sensitive ER calcium release [60]. This evidence suggests that Tat activates a metabotropic receptor and that inositol signaling is central to Tat-induced neurotoxicity. However, Tat toxicity is also related to glutamate receptor activation, since antagonists of NMDA and non-NMDA receptors partially protect neurons from the toxic effects of Tat [62,63,95,118]. Neurotoxic effects of Tat are in part mediated by direct interactions with a polyamine-sensitive site on the NMDA receptor [131,142].

Tat can induce markers of oxidative stress. Striatal injections of Tat caused an increase in protein carbonyl formation, preceded Tat-mediated astrogliosis [2], and caused a loss of striatal tissue in rats [10]. We have shown Tat induced protein and lipid peroxidation in synaptosomal membranes and neuronal cell cultures [128].

Tat-induced neurotoxicity requires interaction of Tat with the neuronal cell membrane [35]. Tat specifically binds to rat brain synaptosomal membranes with moderate affinity (Kd of 2 μM) [135]. When Tat is introduced intracellularly into neurons through patch recording pipettes, it does not alter neuronal membrane potentials [35]. Tat has been shown to bind to the low-density lipoprotein receptor related-protein (LRP) on neurons, internalized, and transported into the neuronal nuclei in a biologically active form [92]. We demonstrated that after cleaving heparin sulfate, a protein required for Tat uptake by the LRP receptor [92], Tat-induced oxidative stress, as measured by protein and lipid oxidation, increased in synaptosomal membranes [128]. Tat-mediated, HIV-infected neurotoxicity is directly evident from a study that demonstrated cell death was completely prevented when the supernatant from HIV-infected monocytes was first immunoabsorbed with antisera to Tat and the HIV-1 protein gp120 [158]. The above findings suggest that Tat is capable of directly exciting neurons and causing excitotoxicity.

Neurodegeneration in HIVD occurs in uninfected neurons at sites that are often distant from the site of viral replication. C6 rat glioma cells stably producing Tat were injected into rat striatum or hippocampus. Bruce-Keller and colleagues demonstrated that Tat could be transported along anatomical pathways from the dentate gyrus to the CA3/4 region of the hippocampus and from the striatum to the substantia nigra, resulting in behavioral abnormalities, neurotoxicity, and reactive gliosis [17]. This finding demonstrates the ability of Tat to cause neuroglial dysfunction at sites distant from that of viral replication by neuronal transport and supports the participation of Tat in HIVD.

The CSF has three main functions: to cushion the brain, deliver nutrients through out, and remove waste from the brain. CSF is produced in the choroidplexus in the lateral ventricles in the middle of the brain. It flows from the lateral ventricles through the interventricular foramen to the third ventricle through the cerebral aqueduct to the fourth ventricle. It exits the fourth ventricle into the spinal cord and subarachnoid space, which cushions the outside of the brain, and is drained by series of sinuses into the venous bloodstream. Extracellular Tat can be cleared from the brain extracellular fluid by the CSF. The movement of the CSF throughout the brain and into the spinal cord offers another explanation of how Tat can cause neurotoxicity and dysfunction at sites remote from viral replication, although significant neuronal death in HIVD patients is not found throughout the brain, but concentrated in the hippocampus and striatum. Extracellular Tat also causes microglia and microphage activation and migration, resulting in neurotoxicity by cytokine and chemokine dysfunction. The ability of Tat to cause neuroglial dysfunction at sites distant from that of viral replication can occur by many different pathways.

7. Indirect neurotoxicity of Tat

Tat stimulates the production of the cytokines, tumor necrosis factor (TNF-α), interleukin 6 (IL-6), IL-8, and IL-10, in glial cells and macrophages [34,119]. These cytokines are released extracellularly and can trigger degenerative processes and apoptosis in neurons. TNF-α has been implicated in stimulation of HIV-1 replication in chronically infected cells [166]. TNF-α releases the excitatory amino acid glutamate from astrocytes [12] and restricts glutamate uptake by astrocytes [54]. This leads to accumulation of glutamate in the vicinity of neurons, hyperactivation of NMDA receptors, influx of calcium, and subsequent cell death. Recently, HIV-1 and gp120 have been shown to impair the ability of astrocytes to transport glutamate related to a defect that inhibits transcription of the EAAT2 glutamate transporter gene [161]. Glutamate levels are increased in CSF and plasma of patients with HIVD compared with HIV patients without dementia and glutamate levels correlate with cognitive decline and brain atrophy [53], although, others did not find any correlation between glutamate levels in CSF and HIVD or HIV infection [47].

Tat activates astrocytes and induces the expression of inducible nitric oxide synthase (iNOS) [93], leading to the
overproduction of nitric oxide (NO), which can react with superoxide anion (O$_2^-$) to form neurotoxic peroxynitrite (ONOO$^-$). TNF-α, which is induced by Tat, also induces iNOS leading to increased production of NO in HIV-infected macrophages [19]. Excess NO also enhances glutamate release from astrocytes [9], adding to NMDA excitotoxicity. Overproduction of NO is proposed to increase HIV-1 replication, as reported in many studies, while low levels of NO inhibit HIV-1 replication [157].

Tat and TNF-α, which is induced by Tat, can stimulate the production of quinolinic acid (QUIN) from macrophages [65,146]. QUIN is a neurotoxin that activates the NMDA receptor raising intracellular calcium and leading to cell death [151]. Elevated concentrations of QUIN are found in the CSF and brains of HIVD patients [65]. Tat induces interleukin-10 (IL-10) production from monocytes [11] and increases mRNA levels of IL-6 [170] by a PKC-dependent pathway. IL-10 is a highly immunosuppressive cytokine that is associated with the disease progression toward AIDS, and IL-6 is increased in HIVD brain [144,162].

A schematic drawing of some of the potential direct and indirect neurotoxic pathways of Tat is shown in Fig. 1.

8. Tat and apolipoprotein E

Apolipoprotein E (apoE) is a small secreted protein of ~34 kDa and a component of several different types of lipoproteins [96]. ApoE is well known for its role in lipid and cholesterol homeostasis, but has also been demonstrated to have immunomodulatory properties in vitro and may regulate smooth muscle and endothelial cell growth and differentiation [86]. The brain is a major site of synthesis for apoE, produced primarily by astrocytes.

In humans, three predominant isoforms of the apoE protein exists, apoE2, apoE3, and apoE4, and are thought to have varying degrees of antioxidant properties [87,111]. The three alleles for human APOE have differential antioxidant capabilities, E2 > E3 > E4 [87,111], and the reverse order displays increased injury from stroke, head injury, and amyloid β-peptide-induced toxicity in brain [34,87,143]. Inheritance of the e4 allele of the APOE gene has been implicated as a major genetic risk factor for late-onset Alzheimer’s disease [100,152]. There are conflicting reports concerning the inheritance of APOE4 and the development of HIV-dementia. Twice as many HIV patients that carry the apoE4 protein were demented or had peripheral neuropathy compared to apoE4-negative HIV patients. In contrast, Dunlop and colleagues reported no correlation in HIV-dementia or encephalitis in relation to APOE genotypes [44]. Recently, it has been demonstrated that there are increased HNE levels, a marker of lipid peroxidation, in brain and CSF of HIV patients with dementia vs. HIV patients without dementia [159], and this finding correlates with the e4 allele of APOE (A. Nath, Department of Neurology, Johns Hopkins University, personal communication). Further, elevations of sphingomyelin, ceramide, and cholesterol was found in the medial frontal cortex, parietal cortex, and cerebellum of HIVD patients with an APOE3/4 or APOE4/4 genotype compared with HIVD patients with an APOE3/3 genotype. There was no difference in the number of astrocytes or activated microglia in any brain region of the two patient populations, suggesting that modification of lipid metabolism in HIVD patients with an APOE4 genotype was not the result of

Fig. 1. Schematic drawing of potential neurotoxic mechanisms involving the HIV protein Tat. Activated microglia and macrophages release Tat, cytokines TNF-α, IL-6, IL-8, and IL-10, and excitotoxins quinolinic acid, which have neurotoxic effects on neurons and activate astrocytes.
increased CNS inflammation [40]. We have shown that human apoE3 acts as an antioxidant, whereas, human apoE4 and mouse apoE, which is similar to human apoE4, do not provide protection against Tat-induced toxicity [128].

9. Antioxidants and Tat

Glutathione (GSH) is the major cellular thiol participating in the maintenance of cellular redox status of the neuron and neuronal mitochondria [28]. A decreased level of GSH may severely impair normal cellular functions. The biosynthesis of glutathione may be compromised by Tat. Tat plays a major role in the glutathione system as evidenced by downregulation in the liver of tat-transgenic mice expression of the γ-glutamylcysteine synthetase regulatory subunit, driving the glutathione cycle towards feedback inhibition, stopping glutathione synthesis, and downregulating the activity of glutathione synthetase [36]. We hypothesize that chronic inflammation of the CNS, activation of microglia, and increased lipid and protein oxidation, all observed in HIV-infected individuals, can lead to the decrease of glutathione levels and potentially HIV-dementia.

In vitro, GSH inhibits viral replication in human monocye-derived macrophages and lymphocytes [66,74,109]. HeLa cells expressing Tat were found to have decreased glutathione peroxidase activity and mRNA levels [133], and expression and mRNA of Mn superoxide dismutase [55]. Protein oxidation was increased and total cellular sulfhydryl content was decreased in HeLa-Tat cells reflecting ongoing oxidative stress [55].

D609 (tricyclodecan-9-yl-xanthogenate) is a selective inhibitor of phosphotidylcholine-specific phospholipase C [112,139]. D609 also displays antiviral activity by inhibiting the shedding of infectious HIV into tissue culture medium from chronically infected lymphoma cells and inhibiting HIV replication, although HIV-specific proteins accumulated intracellularly [4,106]. D609 has been reported to protect against oxidative damage induced by ionizing radiation [169]. A recent study from our laboratory reported the glutathione-mimetic properties of D609 [88]. D609, like glutathione, prevented acrolein-induced alterations of synaptosomal membrane proteins, formed a disulfide (a dixanthate) upon oxidation, and the dixanthate was reduced back to xanthate by glutathione reductase, an enzyme that converts oxidized glutathione to GSH [88]. Moreover, D609 completely protected neurons against the oxidative stress and neurotoxic properties of amyloid β-peptide(1–42) [154]. We demonstrated that primary cortical rat neurons pretreated 1 h with 25 μM D609 and then treated with 500 nM Tat were completely protected against protein and lipid oxidation as measured by protein carbonyls and HNE levels, respectively (Figs. 2 and 3). D609 also protected against Tat-induced TNF-α production (Fig. 4), a cytokine, which is also toxic to neurons, as mentioned above. We also showed that by sequestering intracellular calcium with BaptaAM, Tat-induction of lipid

Fig. 2. D609 attenuates Tat-induced increased protein oxidation. Pretreatment with 25 μM D609 protected primary cortical rat neurons from 500 nM Tat-induced increased protein oxidation. N = 4, *P < 0.01.

Fig. 3. Tat-induced increase of lipid oxidation was protected by pretreatment with D609 and BaptaAM. Pretreatment with 25 μM D609 or 2 μM BaptaAM, an intracellular calcium chelator, protected primary cortical rat neurons from increased HNE, a lipid peroxidation product. N = 4, *P < 0.015.

Fig. 4. Tat-induction of TNF-α is blocked by D609 and BaptaAM. Pretreatment of neuronal cell cultures with 25 μM D609 or 2 μM BaptaAM protected primary cortical rat neurons from Tat-induced increase of TNF levels. N = 4, *P < 0.03.
peroxidation and TNF-α was abolished. D609 may be blocking Tat-induced toxicity, by inhibiting the PC-PLC-specific pathway and/or by acting as a GSH-like antioxidant to block the toxic effects Tat induces through other signaling pathways. Potentially, D609 may be a novel treatment for HIVD.

Estrogen deficiency has been implicated as a risk factor in the development of several neurodegenerative diseases [98,145], and estrogen replacement may result in improvement of cognitive function [7]. Plasma estradiol levels are lower in HIV-infected women [57]. Estrogen has been demonstrated to be protective against oxidative stressors. Pretreatment with 17 beta-estradiol dramatically blocked the activation of NF-κB in human endothelial cells exposed to Tat [89]. In addition, 17 beta-estradiol selectively inhibited the Tat-induced expression of IL-1beta. These results suggest that estrogen may protect against Tat-induced inflammatory reactions in human vascular endothelium via blocking NF-kappaB-mediated molecular signaling pathways. Estradiol can also suppress the proinflammatory effects of HIV proteins [16]. Plant estrogens, flavonoids, are another attractive possibility as a potential treatment modality for HIVD. We have shown that diosgenin, found in fenugreek and yams, can block both Tat-induced and CSF-induced neurotoxicity [158,159]. An advantage of plant estrogens is that they do not have the same feminizing and cancer promoting side effects as estrogens, yet maintain the antioxidant properties. For example, diosgenin has a long carbon chain attached to the sterol ring that cannot be metabolized in humans to form estrogen. Other plant estrogens worthy of further investigation include resveratrol, found in grape skins, peanuts, and red wine, and genistein, daidzein and quercetin, compounds all found in soybeans.

Other widely available drugs with free radical scavenging properties include diethyldithiocarbamate [91], a Chinese herbal medicine BG-104 [6], ferulic acid [77], 3-[4-(N,N-dimethylamino)benzenetetraureyl]propanesulfonic acid sodium salt (NDBT) [75], 5-aminoisaliclycic acid [76], and S-nitrosogluthathione [132].

10. Protection of astrocytes overexpressing Tat

In patients with HIV-1 infection, significant neuronal loss and dysfunction occurs even though neurons are rarely infected [101,104,121]. The most commonly infected cell types in brain are microglia, macrophages, and to some extent astrocytes, although limited viral replication is produced in astrocytes [80,84,101,121,137]. Astrocytes may serve as a reservoir for the virus inducing neuronal damage by releasing cellular and viral products or loss of neuronal support functions. In HIV-infected astrocytes, the regulatory gene tat is overexpressed [117] and mRNA levels for Tat are elevated in brain extracts from individuals with HIV-1 dementia [164].

The HIV-1 protein Tat released from astrocytes reportedly produces trimming of neurites, mitochondrial dysfunction, and cell death in neurons [33]. Intracellular Tat is not toxic to astrocytes. In fact, Tat produced in astrocytic cell lines was able to protect astrocytes from cellular injury induced by 3-nitropropionic acid (3-NP), a mitochondrial toxin; whereas, HeLa cells expressing Tat were not protected [33]. This finding demonstrates that Tat is a neurotoxin at distant sites while protecting the environment where it is produced.

We treated human astrocytes expressing Tat (SVGA-Tat) and vector controls (SVGA-pcDNA) with the irreversible mitochondrial complex II inhibitor 3-NP. Proteomics analysis was utilized to identify changes in protein expression levels. Actin, heat shock protein 90, and mitochondrial single-stranded DNA binding protein were identified as proteins with increased expression, while lactate dehydrogenase had decreased protein expression levels in SVGA-Tat cells treated with 3-NP compared to SVGA-pcDNA cells treated with 3-NP [130]. We found β-actin, calreticulin precursor protein, and synovial sarcoma X breakpoint 5 isoform A to have increased oxidation in control SVGA-pcDNA cells treated with 3-NP compared to SVGA-Tat cells treated with 3-NP [130]. We also utilized proteomics to investigate protein expression changes in human astrocytes intracellularly expressing Tat (SVGA-Tat). We identified phosphatase 2 A, isocitrate dehydrogenase, nuclear ribonucleoprotein A1, Rho GDP dissociation inhibitor alpha, beta-tubulin, crocin-like protein/calumenin, and vimentin/alpa-tubulin to have decreased protein expression levels in SVGA-Tat cells compared to the SVGA-pcDNA cells. Heat shock protein 70, heme oxygenase-1, and inducible nitric oxide synthase were found to have increased protein expression in SVGA-Tat cells compared to controls by blot analysis. These proteins may be critical in understanding how HIV utilizes astrocytes as hosts, without destruction of the host.

11. Conclusions

The HIV-1 protein Tat directly and indirectly induces oxidative stress in neurons, which may be correlated with the dementia observed in some HIV patients [128,159]. That selected antioxidants are neuroprotective against oxidative stress inducers, including Tat, suggests a therapeutic strategy for treatment of HIV-dementia that involves elevation of intracellular GSH levels. Studies to test this notion in model systems are underway.

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References


