Forum Review

Oxidative Stress and Therapeutic Approaches in HIV Dementia

JOSEPH STEINER,1 NORMAN HAUGHEY,1 WENXUE LI,1 ARUN VENKATESAN,1 CAROLINE ANDERSON,1 ROLLIE REID,1 TANYA MALPICA,1 CHAVA POCERNICH,2 D. ALLAN BUTTERFIELD,3 and AVINDRA NATH1

ABSTRACT

Despite the rapidly increasing incidence of HIV infection worldwide and the increasing prevalence of HIV-associated cognitive impairment, even in patients adequately treated with antiretroviral therapy, currently no effective treatment exists for HIV dementia. A broad range of studies using either brain or cerebrospinal fluid (CSF) tissues from well-characterized patients with HIV dementia, animal models, and in vitro studies from several laboratories using HIV-infected cells or HIV proteins provide overwhelming evidence for oxidative stress in mediating neuronal injury in this patient population. These studies also suggest that patients with apolipoprotein E (ApoE) 4 allele are more susceptible to such oxidative damage. In this review, we provide a critical analysis of these studies, including the few clinical trials that have used antioxidants to treat HIV dementia. We also discuss several novel agents with potent antioxidative properties and provide a rationale for combination antioxidant and neuroprotective therapy. Antioxid. Redox Signal. 8, 2089–2100.

THE HUMAN IMMUNODEFICIENCY VIRUS: STRUCTURE AND REPLICATION

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) belongs to the retrovirus family; “retro” because these viruses have a unique enzyme called reverse transcriptase that converts viral RNA to DNA on viral entry into the cell. Viral replication occurs after proviral DNA is integrated into host cell chromosomal DNA. Broadly, the viral genome encodes for two classes of proteins: structural and regulatory (90).

The structural proteins form the envelope, the core, and the matrix of the virus. Three regions within the HIV genome, env, pol, and gag, encode all the structural proteins. The env gene codes for gp160, which is cleaved to form the two major envelope glycoproteins, gp120 and gp41. gp120 forms the surface spikes on the virion, and gp41 is a transmembrane glycoprotein. The pol gene codes for reverse transcriptase, a protease (PR) that cleaves the polyproteins coded by the pol and gag genes into their active forms and an endonuclease that is responsible for viral integration into the host genome. The gag gene codes for all the core proteins. Regulatory proteins encoded by the viral genome control viral genome expression at the level of either the proviral DNA or the viral mRNA. At least six genes (tat, rev, nef, vif, vpu, and vpr) code for proteins that are involved in the regulation of viral replication. These regulatory proteins are not incorporated into the viral particle but regulate viral replication at multiple sites. For example, Tat, Rev, and Nef are targeted to the nucleus of the cell. However, Nef can also be trapped within the cytoplasm of the cell (e.g., in astrocytes), and Tat may be actively released into the extracellular environment. Some of the structural (gp120, gp41) and regulatory proteins (Tat, Nef, Rev, Vpr) have been shown to cause neuronal dysfunction and/or death and thus may be referred to as virotoxins (91).

Our current understanding of the clinical features and pathophysiologic mechanisms that underlie HIV dementia comes from the study of the clade B subtype of HIV-1, which is most prevalent in North America and Western Europe.

1Department of Neurology, Johns Hopkins University, Baltimore, Maryland.
Departments of 2Neurology and 3Chemistry, University of Kentucky, Lexington, Kentucky.

2089
However, worldwide, nearly a dozen clades have been identified, and the most common HIV-1 clade worldwide is clade C. Epidemiologic, pathologic studies from these regions of the world and pathophysiologic studies using these clades are still not available. However, it is likely that important differences might exist, because viral sequencing analysis suggests that mutations may be present in regions of the virus implicated in the neuropathogenesis of HIV dementia (22, 105).

HIV DEMENTIA: CLINICAL MANIFESTATIONS

HIV infection is the commonest cause of dementia in adults younger than 40 years. Before the availability of combination antiretroviral therapy, HIV dementia was noted in nearly 20% of patients with AIDS and often progressed rapidly over a period of months (86, 93). These patients showed a subcortical dementia, manifesting as psychomotor slowing, due to predominant involvement of the basal ganglia (101). In some patients, parkinsonism, cognitive difficulties, and behavioral abnormalities developed, including psychosis and depression. Because of involvement of the dopaminergic system, these patients had extreme sensitivity to side effects from antipsychotic agents (73, 88). Since the availability of combination antiretroviral therapy, the incidence of HIV dementia has become less, and the clinical manifestations less severe. However, because of improvement in survival rates, the prevalence rates of HIV dementia continue to increase (20). In these milder forms of HIV dementia, cognitive dysfunction is more prevalent.

HIV DEMENTIA IN THE ANTIRETROVIRAL ERA

Combination antiretroviral therapy, also termed highly active antiretroviral therapy (HAART), has become the norm in treatment of HIV-infected patients, and these drugs are often started only when immune suppression first manifests, because prolonged use of these agents can lead to drug resistance. However, the virus enters the nervous system early in the course of the illness (34), and because of the long life span and low turnover of glial cells in the brain, once the virus is integrated in these cells, it cannot be eradicated. Two major classes of drugs available for treatment of patients with HIV infection are the reverse transcriptase (RT) inhibitors and the protease (PR) inhibitors. The RT inhibitors target the action of the virion-associated RT enzyme required for the conversion of the single-stranded viral RNA genome into a linear double-stranded cDNA molecule. After transport of the viral cDNA into the host cell nucleus, the HIV genome is integrated into the host chromosomal DNA. Integrated viral DNA, which encodes all necessary viral products for sustained infection, cannot be eliminated unless the cell itself is killed. PR inhibitors target the HIV-PR enzyme required for the posttranslational cleavage of the Gag and Gag-Pol precursor polyproteins into the viral structural and functional proteins required for the production of mature or replication competent virions. However, the production of early gene products such as Tat, Nef, and Rev is not affected by the PR inhibitors. Of these proteins, Tat is released extracellularly and is capable of inducing a variety of effects on brain cells (89). Thus once the virus has entered the brain, theoretically, neural dysfunction may continue even if the viral load is well controlled by RT and PR inhibitors. This possibility is supported by observations that the amount of macrophage/microglial activation in the brain is similar in HAART-treated patients compared with those from the pre-HAART era (8). No impact of HAART on intrathecal IgG production or blood–brain barrier function is found, as measured by the albumin ratio and IgG index or by contrast-enhanced magnetic resonance imaging (1, 12).

Pathology

The pathologic hallmark of HIV-1 infection of the brain is the presence of multinucleated giant cells in the brain, which are formed by syncytia of HIV-1–infected macrophages (92). HIV-1 infects the perivascular macrophages, resident microglia (43), and some astrocytes (106). Infection of other cell types may also occur but remains controversial. Whereas the infection of macrophages and microglia results in a productive infection with formation and release of viral particles (124), infection of astrocytes may result in viral latency, with small amounts of virus released only on stimulation by cytokines (119). Prominent dendritic pruning (84), loss of synaptic density (49), and neuronal cell loss (48, 83) may occur without infection of neurons, suggesting that neuronal dysfunction maybe indirectly mediated. Interestingly, although HIV-1 causes an immunodeficiency by loss of CD4 cells, the immune profiles within the brain suggest an immune activation, particularly with regard to immune mediators regulated by microglia/macroglia and astrocytes (126). Thus an increased production is found of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1 (127) and chemokines such as monocyte chemotactrant protein-1 (MCP-1) (32, 37). Of these mediators, MCP-1 levels in the cerebrospinal fluid correlate best with HIV dementia (32).

Lipid peroxidation

Analyses of brain tissue and CSF of patients with HIV-1 dementia shows evidence for membrane-associated oxidative stress correlated with disease pathogenesis and cognitive impairment, including increased levels of the cytotoxic lipid peroxidation product 4-HNE (63, 123) (Fig. 1). 4-HNE is produced by the oxidation of polyunsaturated fatty acids. Brain polyunsaturated fatty acids are particularly vulnerable to free radicals, leading to the production of multiple aldehydes with different carbon lengths, including 4-HNE (47). 4-HNE is known to modify covalently cysteine, lysine, and histidine residues on numerous proteins and, by this mechanism, can impair the function of membrane ion-motive ATPases, glucose and glutamate transporters, and other proteins important for normal cellular function. 4-HNE may also directly impair mitochondrial function, leading to a mitochondrial release of reactive oxygen products (primarily superoxide), creating a positive-feedback loop of oxidative
Oxidative stress (97). In experimental models of HIV dementia, we have shown that Tat and gp120 can induce protein and lipid peroxidation by mechanisms that may involve disruptions in sphingolipid metabolism (63, 100).

Dysfunctions in sphingolipid and sterol metabolism

Sphingolipids are a class of lipids derived from the aliphatic amino alcohol sphingosine. Three main types of sphingolipids are known: sphingomyelin, ceramide, and the glycosphingolipids, which differ in substitutions in their head group. Ceramide is an important mediator in signaling cascades involved in cell proliferation, stress response, and apoptosis (59). In the brain and CSF of patients with HIV encephalitis, significant increases in long-chain sphingomyelins and ceramides are noted. As the severity of dementia in these patients increases, sphingomyelin and ceramide accumulate and, with moderate to severe dementia, are associated with significant increases in ceramide and HNE in the medial frontal cortex and cerebellum (63). These findings suggest a temporal pattern of dysfunction in lipid metabolism in which sphingomyelin accumulation is followed by increases of ceramide and HNE production. In cultured neurons exposed to the neurotoxic HIV-1 proteins gp120 and Tat, levels of sphingomyelin and ceramide are increased, followed by increased amounts of the reactive aldehyde HNE. Pharmacologic inhibition of neutral sphingomyelinase (mediates the catabolism of sphingomyelin to ceramide) prevented Tat- and gp120-induced increases of ceramide and HNE and inhibition of serine palmitoyltransferase (catalyzes the de novo synthesis of ceramide) and prevented neuronal death, suggesting important roles for neutral sphingomyelinase in HIV-associated neuronal dysfunction and death (63, 67).

These findings suggest that HIV-1 infection may promote a lipid imbalance in neural cells, resulting in an overproduction of ceramide and consequent cellular dysfunction and death. Dysfunctional lipid metabolism may also perturb protein trafficking in cells. For example, gp120 has a galactosylceramide-binding domain, and other proteins with such a domain regulate membrane trafficking from golgi to lipid rafts, suggesting a potential role for perturbed membrane cycling in HIV-1 dementia pathogenesis (85). Emerging findings suggest that membrane microdomains called lipid rafts play important roles in the pathogenesis of HIV dementia. Lipid rafts are regions of the plasma membrane that have high levels of cholesterol and sphingomyelin. Receptors for many different cytokines and growth factors are concentrated in lipid rafts. Lipid rafts are believed to be portals through which many different types of viruses, including HIV-1, enter cells and may also be regions where gp120 and Tat exert their neurotoxic actions (85).

Protein carbonyls

Protein carbonyl groups are used as a marker of protein oxidation (25). These protein carbonyl moieties result from a direct oxidation of many amino acids such as lysine, arginine, histidine, praline, and threonine, β-cis-sion of the peptide backbone, or from binding of the lipid peroxidation product HNE to proteins (25, 116). Alterations in proteins can lead to aggregation, changes in secondary and tertiary structure, susceptibility to proteolysis, fragmentation, and loss of function. The oxidation of proteins by free radicals may be responsible for damaging enzymes critical in neuronal function (116). In addition, oxidized proteins can be cleaved into smaller fragments that may have access to the CSF (116). We found an increase in protein carbonyls in the CSF of patients with severe dementia (Fig. 2). We have previously shown that HIV proteins gp120 and Tat can cause free radical production, which includes gp120-induced free radical–based oxidative damage to human monocytoid cells and Tat-induced oxidative damage in nerve terminals and neurons (54, 75, 100).

Previous studies also show that levels of quinolinic acid (65) and viral proteins are elevated in the CSF of patients with HIV dementia, both of which cause neurotoxicity via mitochondrial dysfunction (123), and Tat protein causes increased levels of calcium in mitochondria in neurons (75). When injected into rat striatum, Tat causes increased formation of protein carbonyls, followed by astrogliosis (5) and loss of striatal tissue (15).

Nitrosative stress

Nitrated tyrosine residues, which provide evidence of peroxynitrite reaction with proteins, are increased in HIV dementia brains (21). The increase in HIV-induced nitrosylation may be mediated by several mechanisms. The levels of inducible nitric oxide synthase (iNOS) in severe HIV-1–asso-
associated dementia coincided with increased expression of the HIV-1 coat protein gp41. Furthermore, gp41 induced iNOS in primary cultures of mixed rat neuronal and glial cells and killed neurons through a nitric oxide (NO)-dependent mechanism (2). The N-terminal region of gp41, which we designate the neurotoxic domain, induces iNOS protein activity and iNOS-dependent neurotoxicity at picomolar concentrations in a manner similar to that of recombinant gp41 protein (3).

Tat activates astrocytes and can also induce the expression of iNOS (79), leading to the overproduction of NO, which can react with superoxide anion (O$_2^-$) to form neurotoxic peroxynitrite (ONOO$^-$). TNF-$\alpha$, which is induced by Tat, also induces iNOS, leading to increased production of NO in HIV-infected macrophages (24). Excess NO can enhance glutamate release from astrocytes (14), compounding N-methyl-D-aspartate (NMDA) excitotoxicity. Overproduction of NO is proposed to increase HIV-1 replication, as reported in many studies, whereas low levels of NO inhibit HIV-1 replication (120).

**GENETIC SUSCEPTIBILITY TO OXIDATIVE STRESS IN HIV DEMENTIA**

In only 20% of patients with HIV infection does dementia develop, and in some patients, encephalitis may occur without dementia (110). These observations suggest that genetic factors may predispose to neuronal toxicity after HIV infection. In humans, three predominant isoforms of the ApoE protein exist, ApoE2, ApoE3, and ApoE4, and are thought to have varying degrees of antioxidant properties (76). The three alleles for human APOE have differential antioxidant capabilities, E2 > E3 > E4 (76). The E4 allele of ApoE has been associated with Alzheimer disease and with poor recovery after brain injury (72) but not with Parkinson disease (46) or Creutzfeldt–Jakob disease (30). Whereas ApoE4 was not associated with higher risk of developing multiple sclerosis, it was associated with disease activity and accumulation of disability (52). The role of ApoE in pathogenesis of HIV dementia remains unclear. In a previous cross-sectional study, no association between ApoE4 and HIV dementia was found (45); however, a subsequent longitudinal study found that the presence of ApoE4 allele resulted in a twofold increase in the risk of developing HIV dementia (40). Recent studies from our laboratory suggest that HIV dementia patients with an ApoE4 genotype show a dysregulated lipid and sterol metabolism (41). Levels of sphingomyelin, ceramide, and cholesterol were significantly increased in the medial frontal cortex, parietal cortex, and cerebellum of HIV dementia patients with an E3/4 or E4/4 genotype compared with patients with an E3/3 genotype. ApoE4 allele also was associated with an increase in HNE adducts of both lysine and histidine, indicative of increased oxidative stress (121). Maximal increases were present in the frontal lobe (Fig. 1), consistent with previous pathological studies showing prominent neuronal loss in this region (83), suggesting that the increased oxidative stress and neuronal injury may be related. By using human neuronal cultures that had been genotyped for ApoE, we found that whereas TNF-$\alpha$-induced neurotoxicity was independent of the ApoE alleles, HIV proteins and HIV protein plus opiate toxicity were more prominent in the ApoE4 neurons. We found that the ApoE4 cultures had increased mitochondrial dysfunction and decreased levels of glutathione, confirming that increased oxidative stress was present on exposure to HIV proteins (100, 121).

**ENDOGENOUS ANTIOXIDANTS IN HIV INFECTION**

Although not studied in the context of HIV dementia, antioxidant levels in HIV-infected patients are altered, a situation...
OXIDATIVE STRESS IN HIV DEMENTIA

...tion that can lead to increased oxidative stress. The tripeptide glutathione [γ-glutamylcysteine-glycine, glutathione synthase (GSH)], present in millimolar concentrations in the brain, functions as an antioxidant and maintains sulfhydryl groups of proteins in the reduced form. Glutathione protects neurons against reactive oxygen species directly and indirectly, and binds lipid peroxidation products such as HNE, thereby providing neuroprotection (44, 99). Glutathione levels are decreased in HIV patients. Serum glutathione levels and glutathione peroxidase activity were significantly lower in HIV patients than in controls, whereas the lipid peroxidation product malondialdehyde, DNA fragmentation in lymphocytes, and total hydroperoxides were increased (57). The concentrations of GSH and other sulfhydryl compounds are decreased in the blood, liver, and central nervous system (CNS) of HIV-infected patients (29), and low GSH is associated with poor survival in HIV-infected patients, whereas administration of GSH to HIV-infected patients decreases mortality (64).

Tat plays a major role in the glutathione system, as evidenced by downregulation of the γ-glutamylcysteine synthetase regulatory subunit in the liver of tat-transgenic mice, driving the glutathione cycle toward feedback inhibition, stopping glutathione synthesis, and downregulating the activity of glutathione synthetase (31). In vitro, GSH inhibits viral replication in human monocyte-derived macrophages and lymphocytes (69, 87). HeLa cells expressing Tat were found to have decreased glutathione peroxidase activity and mRNA levels (109), and expression and mRNA of Mn superoxide dismutase (53). Protein oxidation was increased and total cellular sulfhydryl content was decreased in HeLa-Tat cells, reflecting ongoing oxidative stress (53). Tat and gp120 have also been shown to decrease glutathione levels in brain endothelial cells (102), whereas Tat and gp41 can cause decreased glutathione levels in neurons (75, 117).

TREATMENT OF HIV DEMENTIA WITH ANTIOXIDANTS

A few antioxidants have been tried in small prospective, controlled studies (Table 1). Unfortunately, the findings have been uniformly disappointing. It is possible that targeting a single molecule or pathway may not be sufficient because of the redundancy in the system. The findings of these studies are summarized here.

OPC-14117: A lipophilic compound structurally similar to vitamin E, OPC-14117 acts as an antioxidant by scavenging superoxide radicals. By using the last observation carry-forward analysis, we found no significant difference in the neuropsychological performance between the groups. However, a trend toward improvement in recall after interference ($p = 0.14$), delayed recall ($p = 0.18$), and timed gait ($p = 0.20$) was noted. The global impression cognitive score (rated as normal, 0; mild improvement, 1; moderate improvement, 2; and severe impairment, 3) showed that in the OPC group, three patients worsened, 11 stabilized, and one improved, whereas in the placebo group, nine worsened, five stabilized, and one improved (nominal $p = 0.03$). No significant differences were noted between the groups in mood by using the Center for Epidemiologic Studies Depression scale and activities of daily living by using three different scales. In this study, two of seven patients taking the drug had an elevation in hepatic enzymes that necessitated a decrease in the dosage of the medication to half the original dose (39).

Selegiline (L-Dopa): This monoamine oxidase-B inhibitor was studied in two separate pilot, double-blind placebo-controlled studies (38, 111). The first study was a phase I and phase II study of 36 patients with HIV minor cognitive and motor dysfunction (MC/MD) and HIV dementia in the pre-HAART era, and showed significant improvement in patients taking selegiline, with the verbal learning total score

### Table 1. Clinical Trials of Antioxidants in HIV Dementa

<table>
<thead>
<tr>
<th>Agent</th>
<th>Patients: no. enrolled/ no. completed</th>
<th>Dosage</th>
<th>Concomitant antiretroviral therapy</th>
<th>Duration of study</th>
<th>Conclusions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPC-14117</td>
<td>P = 15/9 D = 15/7</td>
<td>240 mg/day</td>
<td>NRTI</td>
<td>12 weeks</td>
<td>Trend for improvement in memory and time gait test</td>
<td>(39)</td>
</tr>
<tr>
<td>Selegiline</td>
<td>P = 9, Selegiline = 9, Thiocitrate = 9, Both = 9</td>
<td>N/A</td>
<td>NRTI</td>
<td>10 weeks</td>
<td>Improvement in verbal learning. Trend for improvement in recall and psychomotor speed</td>
<td>(38)</td>
</tr>
<tr>
<td>Selegiline transdermal system</td>
<td>P = 5/4 D = 9/8</td>
<td>1.0 mg/cm × 15 cm² patch</td>
<td>NRTI</td>
<td>10 weeks</td>
<td>Positive effect on neurocognition</td>
<td>(111)</td>
</tr>
<tr>
<td>CPI-1189</td>
<td>P = 21/16 D (high dose) = 22/20, D (low dose) = 22/20</td>
<td>100 mg/day; 50 mg/day</td>
<td>HAART</td>
<td>10 weeks</td>
<td>No effect on neurocognition. Improvement in peg board test at highest dose</td>
<td>(53)</td>
</tr>
</tbody>
</table>

P, placebo; D, drug; NRTI, nucleoside reverse transcriptase inhibitor; HAART, highly active antiretroviral therapy.
and trial 5 recall ($p = 0.007$). No other statistically significant effects were noted, although the direction for treatment effects on other tests or recall (delayed recall, recall after interference, correct recognition) and psychomotor processing (Cal Cap mean choice and mean sequential reaction times, symbol digit, and grooved pegboard) consistently favored selegiline. Further, the improvement in memory scores seen at 4 weeks continued to increase at 10 weeks. These trends are particularly interesting because in the same study, another arm of nine patients received thioctic acid. These patients, when compared with the placebo group, performed significantly worse on verbal test total score ($p = 0.005$) and delayed recall ($p = 0.02$). In contrast to that with selegiline, the direction of the effects of thioctic acid on all other neuropsychological test results was consistently negative.

The second study was a small phase I and phase II study using transdermal administration of selegiline in 14 patients with HIV MC/MD and HIV dementia in the pre-HAART era with significant improvement in delayed recall ($p = 0.03$) and in a test of psychomotor speed, the Grooved Pegboard test with the dominant hand ($p = 0.03$). No differences in safety or tolerability were found between patients taking selegiline and patients taking placebo in both studies. Although its mechanism of action is speculative, selegiline may have a neuroprotective and antioxidant effect by decreasing the production of oxygen-free radicals, or at low doses may serve as an antiapoptotic factor, decreasing neuronal injury and death (28). Alternatively, selegiline may elicit improvement in these patients by specific antagonism of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH)–Siah interactions, which have been found to promote apoptotic cell death (60, 114). GAPDH is S-nitrosylated after NOS activation by cell and oxidative stressors, and this signal confers on GAPDH the ability to bind to the E3-ubiquitin ligase, Siah. The GAPDH–Siah complex translocates to the nucleus, where targets of Siah are degraded, leading to cell death. Thus, selegiline and structurally similar GAPDH ligands, like TCH346 (CGP3466), which can disrupt the GAPDH–Siah interaction, may provide neuroprotection (60). A larger phase II trial was recently completed in the United States, and the data are currently being analyzed. Of note, selegiline is best known as an irreversible inhibitor of monoamine oxidase (MAO), an intracellular enzyme associated with the outer membrane of mitochondria. Selegiline inhibits MAO by acting as a “suicide” substrate for the enzyme [i.e., it is converted by MAO to an active moiety, which combines irreversibly with the active site and/or the enzyme’s essential flavin adenine dinucleotide (FAD) cofactor]. Because selegiline has greater affinity for type B rather than for type A active sites, it can serve as a selective inhibitor of MAO type B if it is administered at the recommended dose. The drug is contraindicated in patients taking opiates and should be used cautiously in those taking other psychotropic drugs.

CPI-1189: A lipophilic antioxidant that scavenges superoxide anion radicals, CPI-1189 has been shown to block the neurotoxicity of gp120 and TNF-α (19, 103). It was tested in a double-blind placebo-controlled trial of patients with HIV MC/MD and HIV dementia on HAART conducted from 1998 to 2000. Improvement in the CPI-1189 group was seen in one test of psychomotor speed, the Grooved Pegboard test of the nondominant hand ($p = 0.01$) but not in other neuropsychological tests (35). Side effects include possible cataract formation, transient hepatic enzyme elevation, and a decrease in mean corpuscular volume (35).

**EXPERIMENTAL APPROACHES TO NEUROPROTECTION IN HIV DEMENTIA**

### Estrogen

Estrogen deficiency has been implicated as a risk factor in the development of several neurodegenerative diseases (82, 115), and estrogen replacement may result in improvement of cognitive function (11). In women with HIV infection, menstrual abnormalities frequently develop with increased cycle variability, polymenorrhea (62), or amenorrhea (33, 50). Plasma estradiol levels are also lower in HIV-infected women (58). Hence our laboratory has investigated the possibility that estrogens may be of therapeutic benefit for patients with HIV dementia. With an *in vitro* model of human neuronal cultures, we found that estradiol in physiologic and easily achievable pharmacologic concentrations can protect against the neurotoxic effects of HIV proteins as well as the combined neurotoxic effects of HIV proteins and drugs of abuse such as methamphetamine and cocaine (122). These observations may have important implications, not only for patients with HIV dementia but for drug-abusing populations with HIV infection as well. In these studies, we found that estradiol protects the mitochondria of neurons in a receptor-independent manner (122) (Fig. 1). Estradiol interacts directly with a subunit of mitochondrial F0F1-ATP synthase/ATPase required for the coupling of a proton gradient across the F0 sector of the enzyme in the mitochondrial membrane to ATP synthesis in the F1 sector of the enzyme (133). This may account for the antioxidant properties of 17β-estradiol. Estradiol can also suppress the proinflammatory effects of HIV proteins (23). Hence further studies are necessary in select populations of women with HIV infection who either have cognitive impairment or are at risk for developing dementia. Because of the potential risk of uterine or breast cancer, estradiol cannot be recommended for wide use in this population (77). Importantly, a recent study that has received wide publicity shows that combined hormonal (*i.e.*, estrogen and progesterone) therapy may be associated with cognitive decline in postmenopausal women (107). It is possible that in a subset of patients, hormonal therapy may be detrimental or that progesterone may have effects that negate the neuroprotective effects of estrogen. Further, estradiol, because of feminizing effects, cannot be used in men or in children. Despite the antioxidant and neuroprotective properties of estradiol, it has not yet been used therapeutically in any neurodegenerative disease. Hence it is important to develop compounds that have the neuroprotective effects of estradiol but not the other side effects. As discussed next, some plant estrogens may fit this profile and hence may be worthy of further exploration.

### Plant estrogens

Flavonoids, the so-called phytoestrogens, are a group of compounds made by plants that have antioxidative and neuroprotective properties and hence may be of therapeutic benefit in patients with HIV dementia. Typically, this class of com-
pounds has weak estrogen receptor–binding properties and so does not have the same feminizing and cancer-promoting effects as estradiol. We recently found that diosgenin, a plant-derived estrogen present in fenugreek and yam, can prevent neurotoxicity by HIV proteins and by CSF from patients with HIV dementia (123). Although diosgenin has known anti-inflammatory properties (130), its antioxidant and neuroprotective properties have not been studied before. Other compounds worthy of further investigation include resveratrol, found in grape skins, peanuts, and red wine, which has the additional ability to protect dopaminergic neurons, as shown in models with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity (56); and genistein, daidzein, and quercetin, compounds found in soybeans. Genistein and daidzein are less effective than quercetin as antioxidants; however, they are all well absorbed and hence have good bioavailability (55). In about 35% of subjects, daidzein gets converted in the gut by microflora to equol, which is a more potent antioxidant (129).

**Epigallocatechin gallate (EGCG)**

The major component of green tea, EGCG, has been shown to have antiviral, antitumorigenic, antiinflammatory, antioxidant, antibacterial, antiproliferative, and neuroprotective effects (6, 80, 81). Its therapeutic potential in Parkinson and Alzheimer disease has been suggested (125). 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 (70). EGCG also inhibits HIV-1 replication by blocking the activity of HIV-1 reverse transcriptase (51). Last, EGCG induces virion destruction by deformation of phospholipids by binding to the surface of the viral envelope (131).

**Polyphenols**

Polyphenols also have antioxidant capabilities. HIV-positive patients who drank fruit and vegetable juices had increased lymphocyte proliferation, which could restore disturbances in T-cell homeostasis (128). Moreover, polyphenols such as curcumin and ferulic acid can induce stress response–protective genes, such as heat-shock protein-32 (heme oxygenase-1) (26, 113). These same polyphenolic compounds have demonstrated antioxidant and neuroprotective properties (42). Curcumin, the major constituent of turmeric and a known, naturally occurring antioxidant, protected against lead-induced damage to hippocampal cells of male Wistar rats, as well as lipid peroxidation induced by lead and cadmium in rat brain homogenate. Possible chelation of lead and cadmium by curcumin was proposed as its mechanism of neuroprotection against such heavy-metal insult to the brain. In addition, the antioxidant curcumin provided significant protection against the oxidative stress–inducing 6-hydroxy dopamine (6-OHDA) toxin in a model of Parkinson disease (132). The antioxidant and protective actions of curcumin, mediated through inhibition of nuclear factor (NF)-κB, cyclooxygenase-2, lipoxygenase, and/or inducible nitric oxide synthase, have recently been reviewed (18).

**Selenium**

Selenium is an essential component of the antioxidant enzyme glutathione peroxidase. HIV-infected patients may have low levels of selenium, which has been associated with high rates of mortality in this population (17, 27). Selenium supplementation increases glutathione peroxidase activity and inhibits TNF-α–induced HIV replication (112). Hence selenium replacement may be beneficial in this group of patients. However, glutathione levels themselves may be decreased in HIV-infected cells, in which case in vitro experiments suggest that selenium enrichment has no added benefit (109).

**Glutathione mimics**

N-acetyl-L-cysteine (NAC) is an excellent source of sulphydryl groups and is metabolized in the body into substances that stimulate glutathione synthesis (71). Although no studies have yet been done in patients with neurodegenerative diseases, NAC is widely available as a mucolytic agent for respiratory illnesses and for treatment of acetaminophen-induced hepatotoxicity (71). A controlled trial of NAC in patients with HIV infection showed a reduction in TNF levels in serum and a reduction in the rate of decline of CD4 cell counts (4). L-2-oxothiazolidine 4-carboxylate is another pro-glutathione drug that has been tried in patients with HIV infection. However, NAC is much more effective in scavenging free radicals and has a much more potent antiviral activity in vitro (104). 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 (98). *In vitro*, NAC inhibits viral replication in human monocyte–derived macrophages and lymphocytes (69, 87). The administration of NAC to HIV-infected patients has been shown to decrease mortality (64). Recently, various NAC analogues including N-(N-acetyl-L-cysteinyl)-3-acetylcysteamine have been shown to increase glutathione and display anti-HIV activity, making them possible therapeutic candidates for HIV infection (96). NAC itself does not cross the blood–brain barrier efficiently, but an analogue AD-4 has been developed that has good penetration and has neuroprotectant properties in a rodent model for Parkinson disease (13) and ameliorates findings in experimental allergic encephalomyelitis (94). The exogenous supplement of compounds that increase concentrations of brain glutathione.

**Minocycline**

The tetracycline-derivative minocycline has demonstrated an impressive neuroprotective profile in numerous models of neurodegeneration, from animal models of ALS, Parkinson disease, Huntington’s disease, multiple sclerosis, and ischemic/tranumatic brain injury (10, 16). The compound has significant antiinflammatory actions and achieves significant brain penetration after systemic administration (36, 118). More recently, Zink and colleagues (134) found that minocycline reduced the severity ofencephalitis, suppressed viral load in the brain, and decreased the expression of CNS inflammatory markers in an experimental simian immunodeficiency virus (SIV) model of HIV CNS disease (134). *In vitro* experiments, minocycline treatment inhibited SIV and HIV replication. These antiinflammatory and neuroprotective actions of minocycline may be linked to suppression of p38 mitogen-activated protein kinase (16).

Minocycline and a number of antioxidant compounds protected mixed neuronal cultures in an oxidative stress assay. In
vitro data from lipid peroxidation and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical-scavenging assays show that minocycline, in contrast to tetracycline, is an effective antioxidant with radical-scavenging potency similar to that of vitamin E. These data suggested that the direct antioxidant activity of minocycline may contribute to its neuroprotective effects in some cell-based assays and animal models of neuronal injury (74).

Miscellaneous antioxidants

Many other antioxidants have been tried for AIDS therapy, including vitamin C, vitamin E, lipoic acid, and β-carotene. Vitamin C suppresses the replication of HIV by reduction of reverse transcriptase activity (61), and vitamin E suppresses the activation of NF-κB (66). We have shown that vitamin E can block neurotoxicity induced by CSF of patients with HIV dementia (123). Supplementation of vitamin C and vitamin E was also found to reduce oxidative stress in HIV infection and produced a downward trend in HIV viral load in a blind HIV study (7). HIV-infected patients supplemented with vitamin 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 (68).

Other widely available drugs with free radical–scavenging properties include diethylthiocarbamate (78), a Chinese herbal medicine BG-104 (9), ferulic acid (95), S-nitrosoglutathione (108), and vitamin E. In a recent study, we examined several novel antioxidants for their therapeutic efficacy against the in vivo neurotoxic effects of CSF from patients with moderate to severe HIV dementia. We found that Euk8, diosgenin, and a selenium-containing compound, ebselen, protected against CSF-induced neurotoxicity (123). In a recent study, we found that of these compounds, only disogenin and selegiline protected against the combined neurotoxicity of opiates and HIV proteins, which may have implications for drug-abusing populations with HIV infection (121). Uric acid is a potent antioxidant; however, its insolvability and lack of its ability to cross the blood–brain barrier has limited its use. However, new water-soluble analogues are being developed that may be of therapeutic benefit (personal communication, M. Mattson, National Institute of Aging, Baltimore, MD)

FUTURE DIRECTIONS

These data demonstrate the significant role that oxidative stress plays in the neuropathology of HIV dementia. It is precisely because of this fact that the numerous antioxidant and neuroprotective treatments described have been evaluated in cell-culture models of cytotoxicity relevant to HIV. Thus, this information may be incorporated into the trial design of new clinical trials for therapeutics for HIV dementia. Because potential neuroprotective therapeutics have been evaluated for their cytoprotective effects against oxidative stress–induced injury, inclusion criteria for subsequent clinical trials may then include patients who demonstrate markers of oxidative stress in their CSF or serum samples. In this case, one can evaluate the effect of potential protective therapies on oxidative stress markers as a surrogate marker for neuroprotective efficacy. Quantitative decreases in the levels of protein carbonyls or HNE adducts in serum and/or CSF samples may serve as a clinical correlate for neuroprotective efficacy in patients.

Based on the antiretroviral utility of the multifaceted HAART therapy, neuroprotective therapies may also benefit from such a multipronged approach. It is possible, and perhaps likely, that protection from the degeneration and disease progression of HIV dementia may require a therapy that targets different aspects of the oxidative-stress insult. Combination therapy, incorporating a potent antioxidant coupled with an agent that modifies downstream signaling pathways such as minocycline, may provide a synergistic neuroprotective effect at lower doses of each agent that neither compound could achieve alone. This type of treatment will likely possess less toxicity, because lower doses of each treatment arm would be used, and provide the potential for a one–two knockout punch to decrease the rate of neurodegeneration, which would ultimately result in a concomitant cognitive decline. Thus, the antiretroviral HAART therapy coupled to an antioxidant and neuroprotective combination drug therapy may ultimately be the best treatment regimen for HIV-infected individuals to combat HIV dementia.

ABBREVIATIONS

4HNE, 4-hydroxy nonenal; 6-OHDA, 6-hydroxy dopamine; ApoE, apolipoprotein E; CNS, central nervous system; cps, counts per second; CSF, cerebrospinal fluid; DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate; EGCG, epigallocatechin gallate; FAD, flavin adenine dinucleotide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GSH, glutathione synthase; HAART, highly active antiretroviral therapy; HIV-1, human immunodeficiency virus type 1; HIVD, HIV dementia; PR, protease; IL, interleukin; iNOS, inducible nitric oxide synthase; MAO, monoamine oxidase; MC/MD, minor cognitive and motor dysfunction; MCP-1, monocyte chemoattractant protein-1; MFG, midfrontal gyrus; Mod-S, moderate to severe; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAC, N-acetyl cysteine; NC, normal control; NF-κB, nuclear factor κB; NMDA, N-methyl-D-aspartate; NO, nitric oxide; Non-D, nondemented; O.D., optical density; RT, reverse transcriptase; SIV, simian immunodeficiency virus; TNF, tumor necrosis factor.

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OXIDATIVE STRESS IN HIV DEMENTIA

2098

STEINER ET AL.


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