The Impact of HAART on Advanced Brain Aging: Implications for Mitochondrial Dysfunction and APP Processing

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Abstract

Highly Active Antiretroviral Therapy (HAART) has significantly reduced AIDS-related morbidity and mortality. However, the prevalence of HIV-1-Associated Neurocognitive Disorders (HAND) has been on the rise in the post-HAART era. A majority of the side effects of HAART can in part at least be attributed directly, or indirectly, to mitochondrial dysfunction. Indeed the rapid early clinical phase-in of HAART required dose de-escalations secondary to toxicities suggested to be related to drug side effects affecting mitochondria. Central to central nervous system (CNS) function is the amyloid precursor protein (APP), the parent protein from which amyloid-beta (Aβ) peptide is generated. Aβ generation and aggregation as plaques are well known in the age related dementia, Alzheimer’s disease (AD). It has been demonstrated that Aβ is a common feature of the HIV infected brain as well. Further, reactive oxygen species (ROS) production is upregulated by HAART. Importantly, ROS promote β-secretase expression; a mechanism by which HAART may promote cognitive dysfunction, even in immune-competent HIV infected individuals.

Keywords: Beta-secretase; APP; HAART; Amyloid-β; Microglia

Highly Active Antiretroviral Therapy, HIV Infection and Amyloid-Beta (Aβ)

HIV-associated neuroinflammation is known to occur in even in the face of good virologic control with HAART [1]. As part of this neuroinflammation, the HIV itself promotes deposition of the same amyloid-β peptide (Aβ) found in Alzheimer’s disease (AD; for review see [2]). In HIV infected patients, Aβ immunoreactivity has largely been observed predominantly in the neuronal soma, dystrophic axons, and extracellular space [3-5]. Importantly, this Aβ deposition has been correlated with development of neurocognitive impairment [1].

In further support, Xu and colleagues [6] found, upon examination of autopsy brains of HIV Encephalitis (HIVE) and HIV seronegative cases, similar findings. Although intraneuronal Aβ immunoreactivity is also seen in aged control brains, it was significantly increased in HAART-treated HIV brains. Extracellular Aβ deposition was also found in HAART-treated brains from patients with HIV-associated dementia (HAD) but HAART-untreated HAD brains show only intraneuronal Aβ accumulation [6], indicating some mechanistic role of HAART in Aβ deposition. The prevalence of this intraneuronal Aβ staining was about 30-40%, and extracellular Aβ was present in 4-13% of HIV-infected brains, with a significantly higher percentage of extracellular Aβ present in HAART-treated patients [5]. Importantly, Brew and colleagues found cerebrospinal fluid (CSF) Aβ 1-42 and tau levels correlate with HIV-associated cognitive impairment (HAND) [1].

It is possible that extracellular Aβ (eAβ) and intracellular amyloid-beta (iAβ) are present and interact in a cyclic pathway [7,8]. Neuronal loss is a late event in neurodegeneration. Many changes, including synapse dysfunction, electrophysiological properties and morphological atrophy, occur prior to neuronal loss [9]. Although iAβ and its accumulation may be an early event prior to senile plaque and neurofibrillary tangles (NFT), iAβ may alter cellular functions that would subsequently lead to neuronal loss [7].

iAβ is widely detected in neuronal cells and mainly produced by neurons, but glial cells also produce it in the normal human brain [10]. The iAβ accumulation precedes eAβ deposits and plaque formation. In animal models, iAβ accumulation precedes morphological deficits [11,12]. Aβ is generated by the sequential enzymatic cleavage of amyloid precursor protein (APP), and processing may occur within the endoplasmic reticulum (ER) intermediate compartment [13].

There are several hypothetical pathways that may result in iAβ accumulation [7]. First, iAβ may be formed in the ER, recognized as a misfolded protein, and then translocated to the cytosol where it is ubiquitinated and sent to the proteasomes for degradation [14]. Since this degradation process decreases with aging, or medication toxicities, inefficient clearance of Aβ could result in iAβ accumulation. Secondly, secreted Aβ may be internalized into endosomes [15,16], increasing the membrane permeability of lysosomes [17], and thus, promote leaks into the cytosol. Thirdly, iAβ may occur due to passive leakage along any component of the secretory pathway. Fourth, eAβ passively diffuses through the plasma membrane into the cytosol or is actively brought in by surface receptors [18]. Finally, oxidative DNA damage induces iAβ accumulation resulting p53 mRNA increase in the nuclei leading to Bax and caspase-6 activation and subsequent execution of the cell apoptotic pathway [19].

Importantly, cellular toxicity of iAβ may be cell-type specific, because it induces cell death only in human primary neurons, but not in human primary astrocytes, murine neuroblastoma cells (NT2a), LaN1 or M17 cells [19]. It also appears that the Aβ oligomers, but not fibrils, may be the more toxic species [19], and that the iAβ toxicity may be attributed to these Aβ oligomeric forms.
Thus it is not surprising that accumulation of iAβ is correlated with apoptotic cell death. Alterations in axonal structure and transport may account for the iAβ neurotoxicity and its role in memory function. Accumulation of iAβ increases the number of Golgi apparatus elements, lysosomes and lipofuscin bodies in the hippocampus [20], and also leads to axonopathy with the formation of axonal spheroids as well as myelin ovoids.

There are at least two forms of eAβ, high molecular weight insoluble Aβ fibrils that accumulate in the extracellular space as senile plaques [21] and soluble forms of Aβ that correlate with synaptic dysfunction and cognitive decline [22,23] which include: (a) soluble small globular structures of synthetic Aβ termed Aβ-derived diffusible ligands (ADDLs) [24,25], (b) curvilinear structures of protofibrils [26], and (c) Aβ oligomers; especially nanomers and dodecamers [27].

While Aβ oligomers and ADDL do not seem to progress into insoluble fibrils and plaques, they can interact with cell surface receptors or the cell membrane to gain access into the cells, hence contributing to iAβ load. Likewise, the Aβ fibrils, present as insoluble deposits, could reverse into soluble Aβ monomers. The solubilized Aβ may subsequently gain access into the cells via receptor or membrane mediated mechanisms as described if not degraded by the appropriate proteases such as insulin degrading enzyme (IDE) and neprilysin [28].

The positron emission tomography (PET) tracer 11C-labeled Pittsburgh Compound-B (11C-PIB) specifically binds fibrillar Aβ plaques and can be detected [29]. In a recent case-control study, cognitively unimpaired, HIV infected patients had a 11C-PIB scan within 2 years of concomitant CSF studies and neuropsychometric testing. As would be expected, none of the HIV+ participants had fibrillar amyloid plaques as assessed by increased 11C-PiB Mean Cortical Binding Potential (MCBP) or binding potential within four cortical regions [30]; lending further support to the findings of Brew and colleagues [1]. In the following review we suggest it is possible Aβ biogenesis is increased by the upregulation of β-secretase (BACE) through mitochondrial reactive oxygen species (ROS) activity imparted by HAART.

Disruption of Mitochondrial Function by HAART

Highly active antiretroviral therapy (HAART) has significantly reduced AIDS-related morbidity and mortality. However the prevalence of HIV-1-associated neurocognitive disorders (HAND) has been on the rise in the post-HAART era [31-33]. HAART, and particularly the nucleoside reverse transcriptase inhibitors (NRTI) (especially didanosine, stavudine, zalcitabine, and to a lesser extent zidovudine [3TC]), has been positively correlated with mitochondrial dysfunction [34-37]. Mitochondria are key organelles involved in aging, metabolism, cellular detoxification, and mitochondrial dysfunction, resulting in neurologic plaque formation [61]. Levels of BACE - 1 are increased in vulnerable regions of the AD brain, but the underlying mechanisms are not known.

Importantly, it has been demonstrated that ROS stimulate β-secretase expression [62], suggesting a mechanism by which HAART-induced ROS promotes β-secretase transcription, thereby promoting production of pathological levels of Aβ linked cognitive dysfunction in AD which could be applied to HAND. Indeed deposition of Aβ is common feature of HIV infection [5,63,64]. Mitochondrial dysfunction has been observed in postmortem brains of AD patients [65] just as mitochondria.
in HAART-treated HIV-infected patients [66]. Indeed, mitochondrial dysfunction in both AD [67-69] and HAART-treated patients [66,70-74] is characterized by elevated ROS generation [75], decreased electron transport chain activity, most markedly in cytochrome c oxidase, and altered Krebs cycle enzyme activities [32,45,76,77]. It has been suggested that mitochondria play a pivotal role in the irreversible loss of neuronal function and in the neuronal cell death that occurs during the pathogenesis of both conditions [49,78].

Several studies have indicated mitochondria may be a direct target of AD-associated proteins and peptides such as full-length APP, Aβ peptide, tau, and truncated ApoE4 [79-83] just as HAART directly targets mitochondria. APP and Aβ have both been localized to mitochondria, where they may cause a disruption of basic mitochondrial functions including oxidative phosphorylation or protein import [82]; similar to HAART. Complex IV (of the electron transport chain) seem to be a direct target of both Aβ and truncated ApoE4 [80,84] well as NRTI.

**Aging, Chronic HAART Administration and Development of Cognitive Deficits**

Despite this dramatic improvement in AIDS related morbidity and mortality, high rates of HAND continue to be reported [6,85-88]. Indeed HAND, chronic HIV infection, and aging may all possibly contribute to the development of new forms of neurodegenerative processes based on mitochondrial dysfunction, ensuing upregulation of BACE1, which in turn promotes amyloidogenic APP processing and formation Aβ plaques. All of this would be reflected in accelerated aging-like neurocognitive deficits. The life span increase imparted by HAART also brings patients to an age in which AD is more common and the development of adverse effects of long term medication with HAART may present [89,90].

In support, we recently found that antiretroviral compounds might increase Aβ generation and decrease its clearance by inhibiting microglial phagocytosis, affecting both, amyloidogenic fronts, generation and clearance [90]. Specifically, we found high levels of Aβ42, peptide remaining in the cultured media after N9 microglial cells were treated with antiretrovirals alone or in combination upon completion of phagocytosis assay [90]. In addition, a majority of the compounds tested also significantly reduced levels of phagosomal (cell associated) Aβ 1-42 treated with antiretrovirals alone or in combination upon completion of 42 peptide remaining in the cultured media after N9 microglial cells were generation and clearance [90]. Specifically, we found high levels of Aβ1-42 microglial phagocytosis, affecting both, amyloidogenic fronts, might increase Aβ generation and decrease its clearance by inhibiting oxidative phosphorylation or protein import [82]; similar to HAART. Complex IV (of the electron transport chain) seem to be a direct target of both Aβ and truncated ApoE4 [80,84] well as NRTI.

**Neuroinflammation** is also a feature of both aging and AD (for review see [49,78]). Despite this dramatic improvement in AIDS related morbidity and mortality, high rates of HAND continue to be reported [6,85-88]. Indeed HAND, chronic HIV infection, and aging may all possibly contribute to the development of new forms of neurodegenerative processes based on mitochondrial dysfunction, ensuing upregulation of BACE1, which in turn promotes amyloidogenic APP processing and formation Aβ plaques. All of this would be reflected in accelerated aging-like neurocognitive deficits. The life span increase imparted by HAART also brings patients to an age in which AD is more common and the development of adverse effects of long term medication with HAART may present [89,90].

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Finally, considerable neuroinflammation coupled with mononuclear phagocyte activation has been found in HAART medicated brains, particularly in the hippocampus. Anthony and colleagues [97] found a high level of microglial/macrophage activation that is comparable with the levels seen, pre-HAART, in HIV and AIDS cases. This result was maximal in the hippocampus where microglial/macrophage upregulation in the HAART-treated group exceeded that seen in HIV. In the basal ganglia, HAART-treated cases showed significantly higher levels of CD68-positive microglia/macrophages than in control brains, and in the hippocampus levels were significantly higher than those seen in control cases, pre-HAART AIDS, and presymptomatic brains. Overall there is a significant degree of ongoing neuroinflammation in HAART-treated patients, particularly in the hippocampus. This may pose a threat for the future health of individuals maintained long-term on HAART therapy. [97]. Neuroinflammation is also a feature of both aging and AD (for review see [98]). We and others have shown this resultant elevated secretion of pro-inflammatory cytokines including IFN-γ, TNF-α, and IL-1β can increase Aβ generation and reduce Aβ clearance [6,98,99,100].

In summary it is clear that at least certain HAART regimens, especially those containing EFV, have the potential to cause cognitive decline, despite good control of the HIV itself [87]. Further, it is known that CNS Aβ production is a common feature of the HAART treated brain [3,5] which correlates with cognitive deficits [1]. Therefore, as the aging and efficaciously treated HIV-infected population continues to grow, there will likely be a need to phase in less toxic HAART regimens [101] and/or develop adjunctive neuroprotective, or prophylactic treatments for these undesirable side-effects.
References


