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

Julia Campos de O’Leary1, Demian Obregon1,2,3, Frank Fernandez1,2, Jun Tan1,2,3 and Brian Giunta1,2*

1Neuroimmunology Laboratory, Department of Psychiatry and Behavioral Neurosciences, Morsani College of Medicine, University of South Florida, Tampa, FL, USA
2Rashid Laboratory for Developmental Neurobiology, Department of Psychiatry and Neurosciences, Morsani College of Medicine, University of South Florida, Tampa, FL, USA
3James A. Haley Veterans’ Administration Hospital, Tampa, FL, USA

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-un-treated 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 transllocated 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 neuroblastomas 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.

*Corresponding author: Brian Giunta M.D., Ph.D, Neuroimmunology Laboratory, Department of Psychiatry and Behavioral Neurosciences, Morsani College of Medicine, University of South Florida, Tampa, FL, USA, E-mail: bgiunta@health.usf.edu

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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 (MCPBP) 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) the nucleoside reverse transcriptase inhibitors (NRTI) (especially didanosine, stavudine, zalcitabine, and to a lesser extent zidovudine), have traditionally been suggested to be major culprit in HAART-induce mitochondrial toxicity due to their ability to inhibit Pol-γ, the DNA polymerase responsible for the synthesis of mitochondrial DNA [34,39,40]. Nevertheless, accumulating evidence points to a more complex relationship between these organelles and NRTIs, as well as non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as efavirenz (EFV) and Protease Inhibitors (PI). The rapid early clinical phase-in of HAART required dose de-escalations secondary to toxicities suggested to be related to drug side effects on mitochondria [38]. For example, it has been shown the HAART drug combination of zidovudine (AZT) and the PI, indinavir (IDV) can disrupt the function and viability of endothelial cells due to loss of mitochondrial membrane potential; partially reversible with the thiol antioxidant N-acetylcysteine amide [32]. In adipocytes from HAART treated patients, it has also been shown that NRTI administration correlated positively with mitochondrial DNA depletion [41,42] suggesting an etiology for the lipodystrophy imparted by HAART. There are also coherent experimental and clinical arguments for the existence of mitochondrial toxicity following perinatal exposure to AZT, alone or in combination with the NRTI 3TC [43,44]. Further it has been demonstrated that placentual tissue of HIV-1-infected HAART-exposed pregnancies undergoes mitochondrial DNA depletion with secondary respiratory chain compromise [45] and also that HAART treated pregnant mothers can have children with mitochondrial dysfunction [46]. It has also been found in synaptosomes and isolated mitochondria, as well as human subjects [47,48] that the NRTI, didanosine, can induce oxidative stress, cause the release of cytochrome c, reduce the levels of anti-apoptotic proteins, and increase the levels of pro-apoptotic proteins [49].

**Elevation of ROS, APP Processing and Aβ Biogenesis**

Central to CNS neural 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 the hallmark pathology of Alzheimer’s Disease (AD; [15,50-53]). The peptides have been evidenced to be neurotoxic, as they are reported mediators of inflammation [54,55], and oxidative stress [56]. Aβ peptides are produced via the amyloidogenic pathway of APP proteolysis, which involves the actions of β and γ-secretases [15]. Initially, β-secretase (BACE) cleaves APP, creating an Aβ-containing carboxyl-terminal fragment known as β-C-terminal fragment (β-CTF) [57,58]. In the human brain Aβ1-42 is the predominant form whereas Aβ40 represents about 10% Aβ in the brain and has a greater propensity to form neurotoxic oligomeric and aggregated species (for review, see [59]). NFT, like amyloid, have also been implicated as a central pathological feature of AD. They are misfolded and hyperphosphorylated tau, a microtubule formation protein element (for review see [60]). The accumulation of Aβ can adversely affect discrete molecular pathways, thus facilitating tau phosphorylation, aggregation, and accumulation of abnormal hyperphosphorylated tau. Aβ abnormal hyperphosphorylated tau synergize to accelerate neurodegenerative mechanisms involved in aging, metabolism, cellular detoxification, and mitochondrial dysfunction, resulting in neuritic 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...
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 turns 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 antiretroviral compounded treatments for these undesirable side-effects. 

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 Aβ_{42} 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} suggesting that HAART can cause microglial phagocytosis inhibition [90]. The most significant amyloidogenic effects were observed with combined HAART, suggesting certain HAART medications may have significantly amyloidogenic effects when combined [90]. Recent clinical studies [87,91] further suggests that in well controlled HIV infection, HAART can have a negative effective on cognitive function. It might also be due to limited power. As it has been suggested that earlier initiation of HAART may improve clinical outcomes, the effect of HAART vs. that of unchecked HIV replication on cognitive function will require further prospective studies [91,96].

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.


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