Could antiretroviral neurotoxicity play a role in the pathogenesis of cognitive impairment in treated HIV disease?

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Whilst effective antiretroviral therapy is protective against the more severe forms of HIV-associated brain disease, there remains a large burden of clinically symptomatic cognitive impairment in the modern era. Although several potential pathogenic mechanisms have been proposed, the underlying pathology remains elusive. In this review, we summarize the evidence describing neuronal toxicity of antiretroviral agents themselves in both preclinical and clinical situations, as well as the potential pathological mechanisms underlying this toxicity. We also consider the implications for future practice and clinical research in which case determining optimal antiretroviral combinations that effectively suppress HIV replication whilst minimizing neurotoxic effects on the central nervous system may become paramount.

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suggest the presence of HIV-associated cognitive impairment to be associated with poorer quality of life, poorer clinical outcomes and a higher mortality [5,6].

Although work assessing the role of many of the above pathogenic mechanisms has been undertaken, to date, there has been little focus on the potential CNS toxicity of antiretroviral agents, despite this being a potentially modifiable risk factor for cognitive impairment. Given the dramatic decline in the incidence of HAD since the advent of cART, there can be little doubt that in general cART is beneficial in preventing the more severe forms of HIV-associated neurological disease. However, data describing the role antiretroviral toxicity may play in the pathogenesis of the more minor forms of cognitive impairment remain sparse. Here, we review mechanisms by which antiretroviral agents may cause neurotoxicity, and also review in depth the key, recent data reporting antiretroviral-associated CNS toxicity. Lastly, we consider the implications these data have on the direction for future studies and potential consequences for current clinical practice.

Potential mechanisms

Conceptually, antiretroviral CNS neurotoxicity may be mediated by either direct or indirect mechanisms. The main focus of this review will be on the evidence for direct neurotoxicity from both in-vitro and in-vivo models.

Indirect toxicity

Indirect mechanisms whereby toxicity from antiretroviral agents may develop include drug effects on the CNS mediated by vascular mechanisms and accelerated brain ageing. Vascular disease is an important cause of cognitive decline in PLWH. Reports have suggested that cognitive impairment in otherwise optimally treated HIV infection is associated with prior cardiovascular disease (CVD), as well as hypercholesterolaemia [7]. Some of these effects may be mediated by the unfavourable metabolic consequences of particular antiretroviral agents and combinations; however, the current evidence linking such effects is limited [8].

‘Accelerated ageing’ and ‘frailty phenotypes’ have been reported to occur prematurely in treated HIV disease, with recent work describing a relationship between antiretroviral drug exposure and markers of cellular ageing such as telomerase length [9–11]. The increasing burden of cognitive impairment associated with advanced age, which may be accelerated by chronic antiretroviral toxicity, represents another indirect pathogenic mechanism.

Antiretrovirals and direct mitochondrial toxicity

Given the brain’s significant metabolic demands, it is unsurprising that mitochondrial dysfunction plays a central role in the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease [12]. Evidence of depletion of mitochondrial DNA (mtDNA) in the frontal cortex, with a corresponding increase in oxidative DNA damage, has been reported in HIV-infected individuals compared to HIV-uninfected controls from a small autopsy series [13]. The most marked changes were seen in those with HIV-associated cognitive dysfunction, suggesting a possible causative link.

Direct toxicity to peripheral nerves is a frequent side effect associated with the older nucleoside reverse transcriptase inhibitors (NRTIs), particularly the dideoxy-nucleosides. There is emerging evidence, albeit in mice, that this effect is not limited to the peripheral nerves. Chronic administration of NRTIs was associated with a reduction in mtDNA in murine cerebral cortex neurons [14]. The underlying pathological mechanism is thought to be due to partial inhibition of the mitochondrial gamma DNA polymerase by NRTIs and the resultant mitochondrial toxicity [15]. A series of elegant experiments by Payne et al. [16] has delineated the effects of NRTIs on mitochondrial function. They undertook muscle biopsies from 33 HIV-infected adults (21 NRTI-exposed and 12 never exposed to NRTI), as well as 10 HIV-uninfected healthy controls. Defects in mitochondrial oxidative function were measured using cytochrome c oxidase (COX) histochemistry from muscle biopsy samples. No differences were observed between those with HIV infection who were NRTI-naïve compared with healthy controls. In contrast, NRTI-exposed individuals had an increased frequency of COX-deficient muscle fibres (Fig. 1). The severity of this defect was proportional to the cumulative lifetime NRTI exposure, but not to therapy at the time of biopsy, indicating a possible legacy effect of prior NRTI use. The changes observed were comparable to or even exceeded those reported in healthy elderly adults, despite all participants being aged 50 or under. This toxicity was not thought to be secondary to a direct mutagenic effect of NRTIs, but rather a clonal expansion of pre-existing, age-related, mtDNA mutations. Computerized modelling demonstrated the importance of the timing of NRTI exposure. Older individuals were predicted to have a greater proportion of cells harbouring mutant mtDNA prior to the normal ageing process. NRTI exposure at a later age could then potentially ‘amplify’ these pre-existing mutations, leading to greater toxicity in this group. This is consistent with the observation of more frequent clinical manifestations of mitochondrial toxicity in older HIV-infected patients [17]. Reduced COX activity, in addition to other markers of mitochondrial dysfunction, has been reported in those with Alzheimer’s disease as well as in patients with its precursor, mild cognitive impairment, when compared to controls [18]. However, at present, it is unknown whether the observed antiretroviral-associated mitochondrial toxicity in skeletal muscles is similarly present in the human brain, although...
there is evidence of comparable toxicity in murine models [14].

**Laboratory evidence**

**Cell culture models of direct neuronal toxicity**

Several laboratory models have suggested CNS neurotoxicity to be present for many of the antiretroviral agents in current clinical use. In a key study by Robertson et al. [19], neuronal cell cultures from sacrificed rat brains were exposed to several antiretroviral agents at varying concentrations. Extensive cell death was seen only at the highest concentrations of some drugs (notably efavirenz); however, moderate damage, manifested by dendritic beading and loss of dendrites, was also observed. Of concern, for some antiretroviral agents, these were at concentrations that would be expected using standard clinical doses. In addition, changes in mitochondrial appearance and function were frequently observed, although this did not appear to be clearly related to neuronal toxicity. There was also evidence of increased intracellular calcium influx in response to a glutamate challenge in cells pre-treated with more toxic combinations of antiretrovirals, suggesting that glutamate excitotoxicity may play a role in antiretroviral CNS neurotoxicity. This phenomenon has been linked to other neurodegenerative diseases as well as HIV-associated cognitive dysfunction [20,21].

Utilizing rat neuronal cultures, a direct neurotoxic effect of efavirenz and its metabolites has been described [22]. The 8-hydroxy metabolite of efavirenz was reported to be approximately 10 times more neurotoxic than both the parent drug and other common metabolites. In order to demonstrate the clinical relevance of this finding, plasma and cerebrospinal fluid (CSF) concentration of efavirenz and its 8-hydroxy metabolite were assessed in 13 HIV-infected individuals on efavirenz-containing cART regimens. Potentially, neurotoxic concentrations of both efavirenz and its 8-hydroxy-metabolite were measured in these clinical samples, and for the 8-hydroxy-metabolite, this was three times the minimal in-vitro toxic concentration. The mechanism of neuronal toxicity of 8-hydroxy-efavirenz may be secondary to alterations in neuronal calcium homeostasis. The rapid influx of calcium observed after administration of 8-hydroxy-efavirenz could be normalized with the inhibition of L-type calcium channels. This also offered some protection from dendritic injury induced by 8-hydroxy-efavirenz exposure. The damage associated with efavirenz itself and its other major metabolite appears to be mediated by an as yet unknown, calcium-independent mechanism, although a more recent series of experiments in both human and rat neuronal and glial cell lines suggests acute mitochondrial dysfunction [23]. Interestingly, the authors of this series of experiments demonstrated that neuronal cells, from both human cell lines and primary rat cultures, are more vulnerable to efavirenz-induced mitochondrial dysfunction than the glial cells. This disparity is thought to be due to differences in cellular bioenergetics between the two cell types and the ability of glial cells to activate glycolytic pathways to maintain ATP levels in response to a mitochondrial insult. Preferential mitochondrial toxicity of neuronal cells, measured by mtDNA depletion, has also been observed in mice chronically administered NRTIs [14]. However, further investigation is necessary to assess how these observed mitochondrial changes translate into neuronal dysfunction.

**Animal models of direct neuronal toxicity**

Previous experiments have also demonstrated significantly lower brain creatine kinase activity (this enzyme plays a crucial role in the rapid regeneration of ATP in tissues with high metabolic demands like the brain) in several areas of the brain tissue obtained from mice after the chronic administration of two non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine and efavirenz [24].

Building on this work, further evidence of antiretroviral neurotoxicity is reported in both macaque and rat models in which cART was associated with a decrease in markers of synapto-dendritic integrity [25]. Using an in-vitro primary neuronal cell culture model, the same authors reported neuronal death and damage after exposure to saquinavir and ritonavir, but not to stavudine or zidovudine. This toxicity was associated with an increase
in reactive oxygen species (ROS) and the induction of oxidative stress response genes. In addition, pre-treatment with monomethyl-fumarate, a fumaric acid ester, ameliorated the production of ROS in response to antiretroviral drugs, as well as the previously observed measures of neuronal damage, thus hinting at a potential therapeutic target. These experiments add further weight to the hypothesis that antiretroviral drugs demonstrate the potential for neurotoxicity, which is due, at least in part, to mitochondrial dysfunction and perturbations in cellular metabolism.

**Antiretroviral exposure and similarities to Alzheimer’s disease**

Other models of potential toxicity have been reported. In a neuronal cell culture experiment, zidovudine, lamivudine, indinavir and abacavir were associated with increased neuronal beta-amyloid production, as well as a marked decrease in the ability of microglial cells to phagocytose beta-amyloid [26]. Similar changes have been observed in mice and murine neuronal cell lines in association with oxidative stress caused by efavirenz [27]. Amyloid plaques are the primary pathological hallmarks of Alzheimer’s dementia and have been reported in patients with HIV-associated dementia [28]. Another pathological hallmark of Alzheimer’s disease – the neurofibrillary tangle – comprises of accumulated intracellular hyperphosphorylated tau protein [29]. A small histopathological study reported an increased presence of hyperphosphorylated tau in the brains of those with HIV infection compared to age-matched controls [30]. The greatest increases, with an absence of other HIV-related neuropathology, were seen in those who were stable on cART compared to those who were not on antiretroviral treatment. In patients with HIV encephalopathy, such elevated concentrations of hyperphosphorylated tau were not observed, making it unlikely that direct effects of HIV itself account for the changes seen. In another autopsy study by the same group, cART therapy was associated with a higher level of microglial/macrophage activation, comparable to that seen in HIV encephalitis and untreated HIV-infected patients (with and without AIDS-defining illnesses) [31]. This was without the lymphocytic infiltration seen in those with HIV encephalitis and was most marked in the hippocampus, an area not traditionally associated with HIV-associated dementia. These distinct patterns are not easily explained by HIV infection *per se* and may represent the pathological hallmarks of antiretroviral neurotoxicity. These perturbations in intracellular and extracellular protein deposition may simply be other manifestations of a cellular ‘stress’ response, associated with antiretroviral exposure, which may be implicated in HIV-associated cognitive dysfunction.

**Pathophysiology of antiretroviral cellular toxicity**

In summary, there is compelling evidence that antiretroviral drugs are potentially toxic to neuronal cells because of disruption to the mitochondrial function, either by mutagenic effects on mtDNA or by disturbance of oxidative phosphorylation, leading to changes in cellular bioenergetics with increased production of ROS. This oxidative stress causes induction of multiple inflammatory pathways resulting in cellular dysfunction [32]. This potentially starts a vicious cycle with ROS and pro-inflammatory cytokines causing additional mitochondrial dysfunction though mtDNA damage and impairment of the electron transport chain. ROS from mitochondrial dysfunction has also been shown to produce amyloid beta which itself causes mitochondrial dysfunction and ROS exacerbating the vicious cycle further [33,34]. In addition, this pro-inflammatory milieu activates microglia and astrocytes, which in turn release pro-inflammatory cytokines, further contributing to this vicious cycle and setting up the conditions for chronic inflammation common to many neurodegenerative diseases, including HIV-associated cognitive dysfunction.

**Imaging evidence**

A stumbling block hindering in-vivo imaging work, assessing CNS toxicity of cART, has been the relative bluntness of the tools at our disposal. Standard structural MRI scans are insensitive, and often mild, non-specific changes are all that are observed even in patients with HAD. Advanced imaging techniques show promise, detecting more subtle brain damage, which is likely to be of paramount importance in those with milder cognitive impairment [35].

**Advanced structural MRI analysis**

One study, using a large sample size (*n* = 251), used a semi-automated approach to segment T<sub>1</sub> structural images into cortical and sub-cortical grey matter, white matter and CSF [36]. Regression models identified lower nadir CD4<sup>+</sup> cell count to be significantly correlated with lower volumes of grey and white matter, and compensatory increased CSF volume. Interestingly, longer duration of ART was significantly associated with lower white matter and increased CSF volumes, despite adjusting for other covariates, including duration of HIV infection. Lower white matter volumes have been associated with poorer performance on neuropsychological tests in HIV-infected individuals [37,38] and are clearly an important marker of pathology. One other study examining the relationship between duration of antiretroviral exposure and measures of brain volume [39] did not find a significant association. This disparity may be explained by differences in duration of antiretroviral exposure between the patients in the two studies (mean duration 5.6 years vs. median duration 1.6 years), with a shorter duration of exposure resulting in more subtle and hence statistically insignificant effects.
**1H magnetic resonance spectroscopy**

Cerebral proton magnetic resonance spectroscopy (1H-MRS) allows non-invasive quantification of numerous brain metabolites. Significant reductions in N-acetyl-aspartate (NAA) concentrations, a surrogate of neuronal integrity, have been observed between patients receiving didanosine and/or stavudine compared with those taking zidovudine and lamivudine [40]. This disparity was ascribed to variances in the relative inhibition of DNA-polymerase-gamma, and consequently greater in-vivo mitochondrial toxicity was attributed to didanosine and stavudine.

In a randomized controlled study assessing three different initial cART regimens comprising of a fixed-dose combination of tenofovir and emtricitabine with either efavirenz, ritonavir-boosted atazanavir or zidovudine and abacavir, 30 patients underwent prospective cognitive function testing and cerebral 1H-MRS [41]. In general, improvements in NAA concentrations after 48 weeks’ treatment were observed, and of interest, significant differences were noted between the different drug combinations, with the greatest increase in patients assigned efavirenz therapy. Significant differences were also observed in cognitive function scores, with the greatest improvement in patients assigned the zidovudine and abacavir arm. These results highlight potential differences in neurological outcomes between varying antiretroviral combinations. Longer follow-up (144 weeks) of the same cohort showed the reversal of some of these improvements in cognitive function, as well as worsening 1H-MRS markers of microglial function over time, despite on going successful viral suppression [42]. These data emphasize the potential dichotomy between antiretroviral efficacy and CNS toxicity, providing an avenue for further study. A longitudinal study also reported progressive cerebral injury, measured by 1H-MRS parameters and cognitive function testing, in spite of effective ART [43]. Unfortunately, no control group existed to assess what proportion of these changes were secondary to the normal aging process, although the authors comment that previous data would suggest such an ageing effect to be minimal over the period of study. As HIV replication was controlled and there was no evidence of increased inflammation (by 1H-MRS parameters), the measured deficits may be caused, at least to some degree, by antiretroviral CNS toxicity.

**Functional MRI**

Functional MRI (fMRI) allows the visualization of localized brain activity in real time by exploiting the different magnetic properties of oxygenated and deoxygenated blood, as well as the relationship between brain activity and regional blood flow, so called neurovascular coupling. One study in HIV-infected patients reported the use of ART, when compared to no use of ART, to be associated with poorer performance on more complicated tasks, with increased use of reserve brain networks indicative of reduced cognitive reserve [44]. Differences have also been observed in blood-oxygenation-dependent (BOLD) contrast, the cornerstone of fMRI, between antiretroviral combinations of low and high clinical penetration effectiveness (CPE) score, a surrogate for CNS penetration [45]. The postulated reason for differences between the groups was due to differences in suppression of CNS HIV replication, with lower CPE score leading to poorer suppression and greater subsequent immune activation, with release of inflammatory mediators with vascular activity. CSF HIV-RNA was not measured in this study, so comments on CNS HIV viral replication are only speculative. Unfortunately, only 25% of the participants' plasma HIV-RNA loads were below the limit of detection, making comparisons with effectively treated patients more challenging. One conclusion that can be drawn is that different antiretroviral drug combinations have varying effects on the brain, which may have cognitive implications.

**Clinical evidence**

The majority of clinical research to date has focused on drug penetration and suppression of HIV replication in the CNS [46]. Nevertheless, there have been some clinical studies suggesting ART may be associated with CNS toxicity. Cognitive and psychiatric side effects of medications are well recognized in clinical practice, particularly with efavirenz usage, when early toxicity has been well described. More recently, a more subtle form of neurotoxicity secondary to long-term efavirenz use has been associated with poorer cognitive function in cross-sectional studies [47,48]. Antiretroviral switch studies have reported improvements in general CNS symptomatology when regimens are modified to non-efavirenz-containing combinations [49,50], although these symptoms are often vague and cognitive function was not examined in detail. Interestingly, general CNS symptoms are reported frequently in antiretroviral studies (10% or more) with symptoms including depression and insomnia. Some specific symptoms have been associated with particular antiretroviral agents such as depression with raltegravir use, principally in those taking concomitant medications that would theoretically increase raltegravir plasma concentration [51]. Such findings suggest there may be other clinically relevant CNS toxicities from agents such as raltegravir and warrant further investigation.

**Cessation of combination antiretroviral therapy**

In one study assessing a planned cART interruption, an improvement in cognitive function in patients who ceased medication was observed. This was in contrast to the original hypothesis of the study authors [52]. Since effective cART has been associated with improvements in cognitive function, the results from this study are somewhat surprising. These seemingly paradoxical
findings may be explained by the study lacking a control group, making practice effects difficult to fully discount, and enrolling participants who commenced ART earlier than clinically recommended by some guidelines and before significant immune dysfunction (median nadir CD4+ 436 cells/μl). However, these findings may indicate that antiretroviral neurotoxicity may outweigh the early benefits of viral suppression later on in the course of infection.

**Antiretroviral penetration into the central nervous system and toxicity**

The CPE score was devised to rank antiretrovirals in their ability to penetrate and inhibit HIV replication in the CNS [46]. Conflicting data exist with regards to the role the CPE score for a prescribed cART regimen has on cognitive function. One may expect that if cART was potentially neurotoxic, those drugs with better penetration into the CNS (i.e. have a higher CPE score) would be associated with higher rates of cognitive impairment. In general, the evidence suggests that cART with a higher CPE score has greater suppressive effects on CSF HIV replication. However, the data regarding cognitive outcomes are mixed. Some reports found that regimens with greater CPE scores actually led to poorer cognitive outcomes despite more effective suppression of CSF HIV replication, whilst other studies have shown improvement [53–55]. Discrepancies between different regimes and their ability to suppress HIV replication and improve cognitive function in naïve patients have also been noticed, highlighting the potential differences between efficacy and toxicity, as noted earlier [41].

A large prospective study from six European countries and the United States, comprising nearly 56,000 cART-naïve patients who were followed for a median of 30 months, reported the incidence of HAD to be increased by more than 50% after initiating a cART regimen, with a high CPE score compared to a low CPE score [56]. There were no significant changes in the incidence of toxoplasmosis, cryptococcal meningitis or progressive multifocal leukoencephalopathy, suggesting this effect was not mediated by lack of control of HIV replication or immunosuppression from higher CPE drug combinations. A major drawback is the cohort nature of the study, making definitive conclusions about antiretroviral neurotoxicity impossible.

**Looking forward**

Uncertainty exists regarding the role, if any, antiretroviral neurotoxicity may have on cognitive function (see Table 1 for summary). Although laboratory data suggest such effects exist, teasing such signals out within clinical studies is challenging. Part of this challenge is related to the likely dynamics of cognitive functioning present in HIV-infected individuals commencing ART for the first time. Prior to the initiation of cART, HIV replication in the CNS leads to neuronal dysfunction (via direct toxic mechanisms and immune dysfunction) with consequent effects on cognitive functioning. With the initiation of modern cART, the majority of individuals have HIV replication effectively suppressed. The direct toxic effects of HIV replication are therefore ameliorated and cognitive function may improve. With prolonged suppressive therapy and exposure to potentially neurotoxic drugs for decades, secondary declines in cognitive function may be observed. Consequently, timing is crucial when studying HIV-infected cohorts and assessing

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**Fig. 2. Possible dynamic cognitive function changes over time after initiation of combination antiretroviral therapy.** Hypothetical graph of HIV viral load and cognitive function over time with two hypothetical time points marked. If measured at $T_1$, there will be an improvement in cognitive function relative to $T_0$ (at initiation of cART) due to effective suppression of HIV replication, outweighing any potential neurotoxicity. At $T_2$, there is a clear difference between neurotoxic and neuro-protective cART regimens; however, this is only apparent after a suitable delay. cART, combination antiretroviral therapy.
cognitive function. This principle is illustrated in Fig. 2. Most studies of cART initiation report benefits in terms of cognition; however, follow-up, in common with many treatment trials, is limited to 48 weeks. Accordingly, the benefits of cART on cognitive functioning in the shorter term appear to overwhelm any cART-related neurotoxicity. Longer follow-up, which is rarely undertaken due to cost and logistical issues, is crucial for determining the longitudinal effects of drug exposure and its effects on cognitive function.

The causes of cognitive dysfunction in PLWH are multifactorial and may differ between patients. There is increasing evidence suggesting potential neurotoxicity of several antiretroviral agents in current clinical use. However, the evidence to date for clinically significant CNS neurotoxicity is mixed and far from conclusive. This area is likely to become of increasing interest over the coming years, given that the cohort of PLWH is ageing, leading to an increase in comorbidity and drug–drug interactions with the added complication of age-related changes in the metabolism of antiretroviral drugs (mediated by changes in body mass, renal excretion, p-glycoprotein expression and the presence of polypharmacy amongst others) [57]. Further study is justified within clinical cohorts to identify drugs and combinations of drugs that may be associated with neurotoxicity and to find, where possible, the optimum antiretroviral dose that provides an effective CNS drug concentration which durably suppresses HIV replication, whilst avoiding neurotoxicity.

In conclusion, although there have been many studies assessing cognitive function in PLWH, few have looked specifically at the potentially toxic effects of cART. The evidence so far for antiretroviral CNS neurotoxicity is predominantly from preclinical work and further study is needed to assess the clinical significance of these findings and their impact on the modern manifestations of HIV-associated neurocognitive dysfunction.

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Conflicts of interests

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