High level HIV-1 DNA concentrations in brain tissues differentiate patients with post-HAART AIDS dementia complex or cardiovascular disease from those with AIDS

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Highly active antiretroviral treatment (HAART) has had a significant impact on survival of individuals with acquired immunodeficiency syndrome (AIDS); however, with the longer life-span of patients with AIDS, there is increasing prevalence of AIDS dementia complex (ADC) and other non-AIDS-defining illness, and cardiovascular diseases (CVD) are also common. The influence of these varied disease processes on HIV-1 DNA concentration in brain tissues has not been thoroughly assessed in the post-HAART era. The purpose of the current study is to clarify the impacts of ADC and other complications of HIV disease on the viral load in the brains in AIDS patients with post-HAART. We examined autopsy specimens from the brains of thirteen patients who died from complications of AIDS with quantitative polymerase chain reaction (QPCR). All but one patient had received HAART prior to death since 1995. Two patients died with severe CVD, multiple cerebrovascular atherosclerosis (CVA) throughout the brain and five patients died with ADC. Six patients had no ADC/CVA. A QPCR was used to measure the presence of HIV-1 DNA in six brain tissues (meninges, frontal grey matter, frontal white matter, temporal subcortex, cerebellum and basal ganglia). In the post-HAART era, for non-ADC/CVA patients, HIV-1 DNA concentration in brain tissues was statistically higher than that in patients with ADC. In a new finding, two patients who suffered from severe CVD, especially CVA, also had high concentrations of HIV-1 in brain compartments not showing ADC related changes. To our knowledge, this is the first report of a relationship between the CVA and HIV-1 viral burden in brain. The current observations suggest that HAART-resistant HIV reservoirs may survive within ADC lesions of the brain as well as the macrophage rich atherosclerosis, which needs to be confirmed by more AIDS cases with CVA.

HIV-1, AIDS dementia complex, quantitative PCR, DNA, cardiovascular disease, cerebrovascular atherosclerosis

Before the introduction of highly active antiretroviral therapy (HAART), 20%—30% of patients with late-stage acquired immunodeficiency syndrome (AIDS) developed AIDS dementia complex (ADC). While undergoing HAART therapy, the incidence of ADC has reportedly been cut in half; although with the longer life-span of patients with AIDS, there is increasing prevalence of ADC. It still persists as a significant and debilitating disease. The causes of death for HIV-infected indi-
viduals are numerous and have changed since the introduction of HAART. While a handful of AIDS-defining illnesses still persist (wasting syndrome, non-Hodgkin’s lymphoma, cytomegalovirus disease, *Pneumocystis carinii* infection, atypical mycobacteria infection, brain toxoplasmosis, Kaposi’s sarcoma, tuberculosis, dementia) and other non-AIDS-defining illnesses are also common (hepatitis C infection, non-AIDS-defining lymphomas, cardiovascular disease(CVD), liver dysfunction). In the United States, the life-span of HIV-infected individuals has increased along with the proportion of deaths attributed to non-AIDS-defining diseases[5].

Whether or not non-AIDS-defining illnesses impact the viral burden in the brain is unknown. The most common non-AIDS-defining illness leading to death during HAART is CVD, though the debate as to whether this is a direct result of viral infection or an indirect toxic effect of HAART therapy is ongoing[6−10]. What is clear is that patients with AIDS in the post-HAART era are at a significantly increased risk for myocardial infarction[7], endothelial dysfunction[11], osteonecrosis[12], coronary artery calcification[13], and atherosclerosis[14].

CVD can be present in patients with and without ADC. Sometimes atherosclerotic lesions are identified in the autopsies of patients who died from complications other than CVD. Importantly, these lesions are sometimes found in the brains of both pediatric and adult HIV-infected individuals[15]. As our long-term goals are to identify HIV evolutionary pathways in the central nervous system (CNS) or to define specific viral isolates that may lead to dementia, it was important to first consider CVD as a factor that could impact the viral load and viral sequence genotypes in brain autopsy samples. Additionally the finding that HAART-associated CVD might impact HIV invasion of the CNS would be significant in many areas of research, including epidemiological studies where atherosclerosis in the brain due to CVD, presumably occurring late in infection, would give rise to an entirely different picture of HIV evolution in the CNS.

Most studies of HIV in the brain have relied on pre-HAART patient autopsies. These pre-HAART studies have generated some controversy over the extent of the HIV viral burden in the brain, signature sequences associated with CNS infection, and the compartmentalization of viruses[16−22]. One well-accepted theory is that HIV invasion of the CNS occurs early in infection and evolves independently from the body[23]. In this study we primarily examined HIV-1 DNA in post-HAART CNS tissues and identified a potential process for HIV to enter the brain late in infection via arterial breakdown due to cerebrovascular atherosclerosis (CVA). This finding could impact future studies concerning the mechanisms contributing to post-HAART ADC and the effects of therapy-induced long-term survival on the CNS for those infected with HIV.

1 Materials and methods

1.1 Characterization of patients’ specimens

Frozen specimens from 13 AIDS patients multi-site postmortem autopsies with different neuropathology and systemic diseases were obtained from the UCSF AIDS and Cancer Specimen Resource (Table 1). Standard pathologic definitions of tissue-based diseases were confirmed by pathologic review prior to initiation of genomic DNA extraction of these tissues for quantitative HIV DNA analyses.

Six HAART treated patients had no evidence for ADC but presented with kidney dysfunction, liver dysfunction or HIV-associated viral infections before death. Upon autopsy examination, a small degree of atherosclerosis was identified outside the CNS in one of these patients (ACSR No. 3007235). Two HAART treated patients died with severe CVD (3007234, 3007236). Multiple CVA lesions were identified in the CVD patients; one contained extremely severe atherosclerosis throughout the brain (3007234). Five patients died with medically diagnosed ADC; four of these patients had received HAART since 1995, while one was not (3006990). For all patients that had been on HAART, quantitative polymerase chain reaction (QPCR) was utilized to measure and identify the presence of HIV DNA in 6 brain tissues (meninges, frontal grey matter, frontal white matter, temporal subcortex, cerebellum and basal ganglia). Only three of these tissues were available for the pre-HAART patient examined (meninges, frontal white matter, and temporal subcortex).

1.2 Preparation of genomic DNA

Genomic DNA in different tissues were prepared by QIAamp DNA Mini Kit (QIAGEN company, Valencia, CA) and quantified by absorbance at 260 nm (NanoDrop® ND-1000 UV-Vis Spectrophotometer, NanoDrop Technologies, Wilmington, Delaware).