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Early suppressive antiretroviral therapy in HIV infection is associated with measurable changes in corpus callosum

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INTRODUCTION

Many studies have demonstrated brain injury and cognitive impairment in HIV infection (Kramer-Hämmerle et al. 2005; Ghafouri et al. 2006; Chiang et al. 2007; Cardenas et al. 2009; Chang et al. 2011; Ances et al. 2012; Holt et al. 2012; Jahanshad et al. 2012). Such injury, however, has been observed years after initial viral exposure. Evidence emerging from *in vivo* imaging studies of acute and recent infection indicates that onset of brain alterations may occur soon after infection (Lentz et al. 2009; Ragin et al. 2012; Valcour et al. 2012; Ragin et al. 2013). Consistent with early central nervous system (CNS) involvement, virus can be detected in cerebrospinal fluid (CSF) within one week of HIV infection, and pathologic inflammatory changes in brain metabolites have been quantified in both acute and early infection (Lentz et al. 2009; Sailasuta et al. 2012; Lentz et al. 2011). These findings raise imperative questions concerning the potential neuroprotective benefit of initiating antiretroviral therapy (ART) early in the clinical course. In this analysis, we used high-resolution neuroanatomic imaging together with quantitative analysis capabilities at

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CONFLICTS OF INTEREST

For all other authors, no conflicts of interest were declared.

microstructural (Diffusion Tensor Imaging; DTI) and macromolecular (Magnetization Transfer Ratio: MTR) levels to evaluate the status of the brain in ART-naïve, ART-suppressed (plasma viral load [vRNA] < 50 copies/mL), and seronegative groups. Samples were comprised of well-characterized participants of the larger Chicago Early HIV Infection Study (Ragin et al. 2012). This cohort, spanning acute HIV and early infection (defined as the approximate first year of infection), was established to illuminate the natural history of changes occurring in the brain and is therefore ideally suited for evaluating effects of ART at the earliest stages of neurological injury. While initial findings from this cohort did not identify differences in brain imaging or neurocognitive measures associated with ART use, virologic suppression was not taken into account.

METHODS

Data Source

This investigation included 30 participants from the Chicago Early HIV Infection Study, an ongoing observational cohort of participants infected, on average, less than one year prior to enrollment. Subjects were recruited primarily from the Infectious Disease and Sexually Transmitted Disease Clinics of Northwestern Memorial Hospital. Cohort participants were well-characterized for vRNA, CD4+ cell counts, exposure to ART and duration of infection determined by an early infection assay (EIA; Blood Systems, San Francisco, CA, USA). EIA utilizes a recent infection testing algorithm, which analyzes both the titer and binding avidity of HIV-1 antibodies in concert (measured using a modified third-generation assay) to estimate mean duration of infection (Keating et al. 2012). Briefly, the algorithm developed by Keating et al standardized cutoff values of titer and binding avidity to determine mean duration of recent infection, which in their sample was 146 ± 19.7 days (95% CI 107.4, 184.7). Study exclusion criteria of the Chicago Early HIV Infection cohort included history of neurological disorder or head injury, opportunistic infections, current alcohol/substance dependence, and magnetic resonance imaging contraindications. Further details concerning the cohort and study procedures were detailed in a prior analysis of this cohort (Ragin et al. 2012).

Study Subjects

Of the 55 HIV-infected and 21 seronegative subjects in the Chicago Early HIV Infection cohort, 10 subjects were identified who were ART-exposed and virologically suppressed with vRNA < 50 copies/mL at study entry (ART-suppressed group). For each ART-suppressed subject, ART-naïve HIV-infected subjects with similar duration of infection were identified (as determined by EIA values within approximately ± 5 units). The ART-naïve subject most similar in age to the ART-suppressed subject was then selected. This approach was used to construct a sample of 10 ART-naïve subjects (ART-naïve group). Matching of seronegative controls was based on the average age of the ART-suppressed and ART-naïve pairs. Demographic and clinical information is presented in Table 1.

Brain and Neurocognitive Status Measures

All participants were evaluated with quantitative brain imaging (Ragin et al. 2010). Subjects were scanned at 3 Tesla (Magnetom® Verio; Siemens AG, Erlangen, Germany). SIENAX

(Oxford University, Oxford, UK) was used to calculate measurements for total brain and ventricular volume and for specific tissue classes (Smith et al. 2002). A separate algorithm, Freesurfer, was used to derive measurements of individual regions and landmarks of the brain (Fischl et al. 2002). Semi-automated and automated strategies were used to derive these volumetric measurements as well as DTI (fractional anisotropy: FA and mean diffusivity: MD) and MTR parameters for 3D volumes of interest (VOI) including: cerebral cortex, cerebral white matter, caudate, putamen, hippocampus, thalamus, aggregate corpus callosum (CC), and individual CC segments. MTR and DTI parametric maps were constructed to capture FA, MD and MTR within each voxel of a VOI. Voxel measures were then averaged for each parameter to derive regional FA, MD, and MTR values. Technical details are further described by Wu et al (Wu et al. 2012). A neuropsychological test battery that has been used in large neurological outcome studies in HIV infection (Sevigny et al. 2004) was used to evaluate cognitive function. The battery included tests of verbal and visual memory, visuoconstruction, literacy, frontal executive function, psychomotor skills, and motor speed.

Statistical Analysis

Continuous variables, including imaging and cognitive measures, were compared in the ART-suppressed, ART-naïve, and control groups using analysis of variance (ANOVA). A significance level of 0.05 was used with Tukey adjusted post-hoc comparison. All analyses were executed with SPSS v 21 software (SPSS; IBM, New York, NY, USA).

RESULTS

Subjects were matched by EIA as a surrogate for duration of infection; EIA between the ART-suppressed group and the ART-naïve group were not significantly different ($p = 0.54$). Baseline characteristics, except marijuana use and vRNA, were similar between the ART-suppressed and ART-naïve subjects (Table 1). Volumetric analysis showed significantly higher CSF percent in the ART-suppressed group compared to the ART-naïve group ($p = 0.037$). The lateral ventricle percent was also significantly higher in the ART-suppressed than in the ART-naïve group ($p = 0.007$). Anterior CC measurements, including FA and MTR were significantly lower in the ART-suppressed group compared to controls (FA: $p = 0.05$; MTR: $p = 0.028$). The ART-suppressed group had weaker scores than controls on the Karnofsky Performance Scale ($p = 0.03$) and worse scores on the Beck Depression Inventory ($p = 0.038$). No significant differences were detected among the groups in any other neurocognitive domain. No correlation between the significant imaging and neurocognitive findings was identified. Results are presented in Table 2.

SIENAX and Freesurfer measurements did not detect significant differences in total brain, total white matter, total gray matter, cerebral cortex, or other sub-cortical structures between the three groups.

DISCUSSION

Imaging analysis of specific neuroanatomic regions, landmarks and major constituent tissue classes revealed alterations in the anterior segment of the CC as well as ventricular

expansion in ART-suppressed subjects. The MTR was significantly reduced in the anterior CC of ART-suppressed subjects compared to the seronegative controls. MTR declines with pathological changes to brain tissue as exchange of magnetization by macromolecules in membranes and lipids is altered (Wu et al. 2012). A consistent pattern involving the anterior CC was also quantified in the ART-suppressed group using a different quantitative imaging modality; specifically, loss of white matter integrity (reduced FA) was identified by DTI. There were no significant differences between the CC of ART-naïve subjects and seronegative controls.

Additionally, the CSF percent and lateral ventricle percent were higher in the ART-suppressed subjects compared to the ART-naïve subjects. Ventricular expansion and increased CSF can be interpreted as *ex vacuo* tissue loss in surrounding regions; the lateral ventricles are situated under the CC, making their volumes potentially susceptible to change with alterations in the CC. Many studies of advanced infection in the ART era have confirmed injury in the CC, and thinning of the CC has been observed with associated ventricular expansion by other computational neuroanatomy methods (Wu et al. 2012; Thompson et al. 2006). The observed CC changes and CSF volume expansion in the suppressed group are consistent with a significant loss of CC integrity in the ART-suppressed group.

Initial Chicago Early HIV Infection Study results did not find brain structural differences associated with ART (Ragin et al. 2012). The DTI and MTR measures used in the current analysis, which were not examined in the earlier study, quantify alterations occurring at microscopic levels for 3D volumes of interest for more comprehensive tissue measurement (Wu et al. 2012; Le Bihan 2003). These measurements may detect subtle changes in brain tissue that may not be evident based on net volume. In addition, prior Chicago Early HIV Infection Study analyses did not consider virologic suppression as a factor in assessment of volumetric and neurocognitive endpoints and likely included participants with only brief (thus not virologically suppressive) ART exposure. In this study, all subjects with ART exposure achieved virologic suppression and the comparison was less likely to include participants with only brief exposure or inadequate adherence.

The prevalence of HIV-associated dementia has declined substantially in the contemporary treatment era, however the prevalence of less severe HIV-associated neurocognitive disorder diagnoses has increased (Heaton et al. 2010). This, along with evidence of cognitive improvement with treatment suspension (Robertson et al. 2010), has raised concerns of ART neurotoxicity. The majority of ART recipients in this study (60%) were virologically suppressed on Atripla®, which is a fixed-dose medication that includes efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI). Metabolites of efavirenz have demonstrated potent neurotoxicity *in vitro*, causing damage to dendrites in cultured neurons through dysregulation of calcium homeostasis (Tovar-y-Romo et al. 2012). Many other ART formulations have demonstrated a capacity for neurotoxicity as well; a systematic analysis of *in vitro* effects of 15 antiretroviral compounds, including nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs and protease inhibitors (PIs) on neurons found a wide range of neurotoxic mechanisms, such as mitochondrial toxicity, with the ultimate endpoint of neuronal dysfunction (Robertson et al. 2012). Alternatively, neuroinflammation in the CC

may be secondary to the immune reconstitution following ART initiation, which has been observed in animal models (Mutnal et al. 2013).

As the largest white matter structure in the brain with projections to all major regions and serving a critical role in interhemispheric communication, alterations in the CC may contribute to impairment in various functional domains. The ART-suppressed group reported greater difficulty on the Karnofsky Performance Scale, a functional measure of activities of daily living (Table 2); this group also had the worst scores on the Beck Depression Inventory (Table 2). Persistent CSF viral load is associated with depression, however one would then expect worse depression scores in the ART-naïve group, rather than the ART-suppressed group as we found here (Hammond et al. 2014). Efavirenz has been associated with neuropsychiatric side effects, including depression (Muñoz-Moreno et al. 2009; Celano et al. 2011). Suppressive ART may therefore be contributing to both depressive symptoms and to alterations in the CC. Incidentally, it may be relevant that measurable MTR and DTI changes in the CC have been reported in association with depression (Gunning-Dixon et al. 2008; Benedetti et al. 2011; Cole et al. 2012; Guo et al. 2012; Seok et al. 2013; Xu et al. 2013). Therapeutic ART could directly or indirectly result in CC changes, which in turn may manifest clinically as depression and functional difficulties.

Various potential confounders contributing to these conclusions deserve mention. We were unable to appropriately control for marijuana use owing to the small sample sizes. Cumulative marijuana use has been found to correlate with impaired axonal connectivity in splenium (Zalesky et al. 2008) and heavy, cumulative marijuana use (>2 years) with loss of anterior CC integrity (Arnone et al. 2008). While marijuana use was higher in the ART-suppressed and ART-naïve groups, there were no differences between CC measurements in marijuana users and nonusers among these groups (data not shown).

Furthermore, the ART-suppressed group may have been selected for treatment because of lower CD4+ cell count nadirs or other unreported indicators of more advanced HIV disease. If so, the ART-suppressed could have had more advanced disease pre-ART initiation contributing to the observed differences. While duration of infection was similar, subjects who have lower CD4+ nadir in the early clinical history are at greater risk for neurological progression (Ellis et al. 2011), including brain structural alterations (Jernigan et al. 2011). Early infection is characterized by marked loss of CD4+ cells in association with viremia. Some spontaneous recovery in CD4+ cell counts may occur in both treated and untreated patients at approximately 4 months, however, this is short-lived (Le et al. 2013). Recovery in CD4+ cell counts occurs slowly and may be arrested, even in those who achieve viral suppression on treatment (Le et al. 2013). As shown in Table 1, with average treatment duration of 142 days, median CD4+ cell counts were higher in the ART-suppressed group (443 vs. 370 in the ART-naïve group), reflecting immunologic response to therapy and consistent with anticipated gains of 50 cells/mm³ within the first several weeks following initiation of ART.

Additional limitations of this study are the observational nature of the design and the small samples sizes. Due to the observational approach, pre-existing psychiatric history and extent

of substance abuse history were not identified. Nevertheless, it is unlikely that the findings observed for the anterior CC were due to chance; differences in the anterior CC were detected in the ART-suppressed group by two different imaging modalities. A consistent pattern was obtained for measurements of lateral ventricular volume as well as CSF percent, which were significantly higher in the ART-suppressed group compared to the ART-naïve group. As previously discussed, changes in lateral ventricle volume may be sensitive to CC alterations, further underscoring this CC finding.

Findings from this small sample study cannot be interpreted as conclusive evidence that ART is harmful to the brain. Rather, these findings merit attention as they indicate that early treatment may alter the trajectory of neurological progression in HIV infection. Additionally, it cannot be assumed that the CC changes observed in the ART-suppressed group are injurious; they may reflect ART-associated changes in regions with a predilection for HIV infection and may be associated with long-term benefit. Moreover, they may reveal that specific sub-cortical tissue is more susceptible to inflammation due to a variety of factors, including immunosuppression, viral infection, illicit substances, and therapies like ART.

Tremendous reductions in mortality and disease-associated morbidity in HIV-infected patients have been achieved with suppressive ART (Palella et al, 1998). Early virologic suppression confers profound benefits such as curtailing T-lymphocyte destruction, enhancing immune restoration and limiting expansion of viral reservoirs (Lori et al. 1999; Oxenius et al. 2000; Strain et al. 2005; Brenchley and Douek 2008; Hunt 2005; Zeng et al. 2012; Eriksson et al. 2013; Ananworanich et al. 2013; Le et al. 2013). Adverse effects of ART on various organ systems have also been observed, including neurotoxicity. Despite the limitations to this study, early suppressive ART may contribute to the brain structural alterations and neurocognitive outcomes observed, the enduring result of which remains undefined. As current recommendations of earlier treatment initiation (Department of Health and Human Services, 2013) will result in earlier and longer cumulative exposure to ART, further longitudinal studies will be necessary to examine the long-term neurologic outcome of early suppressive ART, taking into consideration its prevailing established benefits.

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Table 1

Characteristics of study subjects

Subject Characteristics				
	ART-Suppressed	ART-Naïve	Control	p-value
Age in years (mean $\pm$ SD)	31.6 $\pm$ 9.9	32.5 $\pm$ 10.4	34.2 $\pm$ 11.2	0.86
Gender (% male)	80%	70%	100%	0.20
Race				
Caucasian	60%	50%	70%	0.82
African American	40%	40%	20%	0.58
Other	0%	10%	10%	0.61
Education				
High School	30%	30%	0%	0.16
College	60%	70%	70%	0.87
Graduate Education	10%	0%	30%	0.14
Substance Use (past 30 days)				
Alcohol (heavy drinking ^a)	10%	10%	30%	0.41
Marijuana	40%	40%	0%	0.05 ^c
Cocaine	10%	0%	0%	0.37
Heroin	0%	0%	0%	
Methamphetamine	10%	0%	0%	0.37
Other	0%	10%	0%	0.32
NART-R ^b	101.7 $\pm$ 15.6	107.1 $\pm$ 11.5	107.4 $\pm$ 6.5	0.52
Clinical characteristics of the HIV participants				
CD4 cell count, cells/ μ L (Median, range)	443, 139–710	370, 162–1282	---	0.83
Plasma HIV RNA, copies/mL (Mean $\pm$ SD)	37.3 $\pm$ 14.2	85099.1 $\pm$ 130559.0	---	0.05 ^c
EIA assay (Mean $\pm$ SD, range)	41.7 $\pm$ 27.2, 3.94 – 77.45	34.2 $\pm$ 26.0, 4.58 – 76.75	---	0.54
ART duration, days (Mean $\pm$ SD, range)	142.5 $\pm$ 119.1, 35–323	---	---	---
ART Regimen				
Atripla® (TDF/3TC/EFV)	60%	---	---	---
Other	40%	---	---	---

^aHeavy Use defined as >7 drinks/week for women, > 14 drinks/week for men^bNART-R: National Adult Reading Test – Revised^cSignificant p-values

Table 2

Results of quantitative brain imaging and neurocognitive testing

Autoregional Parameters				
	ART-Suppressed	ART-Naive	Control	p-value
Anterior Corpus Callosum	43.351 ± 1.283	44.427 ± 1.679	44.931 ± 0.715	¹ 0.168
Magnetization Transfer Ratio (Mean ± SD)				² 0.028 ^c
				³ 0.660
Anterior Corpus Callosum	0.645 ± 0.0736	0.681 ± 0.0617	0.71 ± 0.0375	¹ 0.368
Fractional Anisotropy (Mean ± SD)				² 0.05 ^c
				³ 0.544
Freesurfer Volumetric Measurements				
	ART-Suppressed	ART-Naive	Control	p-value
Lateral Ventricle Percent (Mean ± SD)	0.0103 ± 0.003	0.00571 ± 0.003	0.0086 ± 0.003	¹ 0.007 ^c
				² 0.430
				³ 0.112
CSF Percent (Mean ± SD)	0.00084 ± 0.0002	0.00067 ± 0.0002	0.000796 ± 0.0001	¹ 0.037 ^c
				² 0.798
				³ 0.137
Neurocognitive Testing Parameters				
	ART-Suppressed	ART-Naive	Control	p-value
Beck Depression Inventory (Mean ± SD)	16.30 ± 13.865	13.43 ± 13.879	3.30 ± 2.541	¹ 0.859
				² 0.038 ^c
				³ 0.173
Karnofsky Performance Scale (Mean ± SD)	93.00 ± 6.749	97.00 ± 6.749	100.00 ± 0	¹ 0.267
				² 0.03 ^c
				³ 0.485

¹ ART suppressed group compared to ART naive group;² ART suppressed group compared to controls;³ ART naive group compared to controls^c Significant p-values