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HIV-1 Infection and Central Monoamine Neurotransmitters

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Abstract: Human immunodeficiency virus type 1 (HIV-1) enters the central nervous system (CNS) shortly after infection and gets localized in different brain regions, leading to various types of neuropathological problems. It has been hypothesized that HIV-1 infection mediated neuropathogenesis may also adversely affect the activity of the central monoamine neurotransmitters systems, such as dopamine (DA), and 5-hydroxytryptamine (5-HT, serotonin), resulting in neurocognitive deficits and mental health problems. However, investigations are scarce with respect to the status of these neurotransmitters in the CNS of HIV-1+ individuals, particularly in those patients who had received antiretroviral therapy (ART) during life. Since, mental health problems and neurocognitive and neuropsychological deficits continue to persist even after ART intervention, it is pertinent to determine the CNS status of the neurotransmitters associated with these functions. We determined the neurotransmitters, dopamine and its metabolite, homovanillic acid (HVA) in different brain regions of a group of autopsied cases of HIV-1+ and HIV-1 negative controls, using highly sensitive CoulArray HPLC-ECD system. Distribution of HIV-1 viral RNA in these brain regions was also measured using real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) technology with high sensitivity of detection (<5 copies of RNA). In a separate study we reported measurement of 5-HT (serotonin) and its metabolite, 5-hydroxyindole acetic acid (5-HIAA) in the cerebrospinal fluid (CSF) obtained during life of neurologically asymptomatic HIV-1 infected patients and controls. A substantial decrease and a wide variation in the concentration of DA as well as its metabolite, homovanillic acid (HVA) was found in the autopsied brain regions of HIV-1 infected individuals. Wide variation was also found in HIV-1 RNA concentrations in different brain regions with no specific pattern observed in any region. Brain regions of HIV- negative individuals showed no detectable viral RNA. Majority of HIV-1+ individuals had demonstrated neurocognitive impairment during life despite ART intervention. A significant decrease in 5-HT concentration was also found in the CSF of HIV-1 infected patients. These studies demonstrate that HIV-1 infection adversely affects the central DA and 5-HT systems.

Key Words: HIV-1, CNS, Monoamine Neurotransmitters

INTRODUCTION

Human Immunodeficiency virus type-1 (HIV-1) infection continues to spread globally. Despite various efforts to contain the pandemic, including the introduction of antiretroviral therapy since 1996, the number of people living with HIV continues to increase every year. Currently, 40 million people worldwide are estimated to be affected by this disease, of which 37 million are adults and 3 million children. Every year about 5 million people are newly infected by the virus, and more than 3 million die because of various complications of illness due to HIV-1 infection and its progression to acquired immune deficiency syndrome (AIDS) (UNAIDS, 2006). Regardless of the global diversity of people with respect to region, country, culture, religion, race, gender, age, life style, mode of infection, or even the risk factors involved in contracting the infection, the health consequences remain common among all people infected with HIV-1.

Moreover, in all people, the mode of HIV-1 infection and the mechanisms involved in the pathogenic consequences are also common, and include opportunistic infections, neurological disorders, neuropsychological dysfunctions, motor disturbances, behavioral changes and neurobehavioral disorders including HIV-associated dementia (HAD). Although, initially the major target of HIV-1 infection is the

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CD4+ T helper cells, the virus also rapidly infects the other cells including monocytes, macrophages and dendrtic cells, further perpetuating the infection, since these cells become the reservoir of actively replicating virus, and an important source of its transport to the other tissues and organs. Although, HIV-1 enters the CNS soon after infection and gets lodged in different brain regions [1], the mode of entry of virus into the CNS is still debatable. The suggested models include; 1) trafficking of virus through the infected monocytes and possibly lymphocytes across the blood brain barrier [2], 2) circulating cell- free virus infiltration in to the brain, and 3) the transfer of virus through the vascular endothelium[3]. The delivery of virus in the brain through the infected macrophages allows the long-term persistence of HIV-1 in the CNS and its preferred localization in the subcortical and frontostriatal circuitaries [4,5]. Although, highly active antiretroviral therapy (HAART) has been effective in decreasing the peripheral viral load, these drugs do not reach the brain in optimal levels and therefore can not reduce HIV-1 replication, making CNS as its sanctuary site [6]. Early reports found that during late in the course of the disease, even in the absence of opportunistic infections, 30-60 % of HIV-1 infected patients, develop a number of neurological problems, including cognitive impairment as well as motor disorders, which slowly progress to AIDS dementia complex (ADC) [7, 8]. The neurological problems and behavioral changes observed in HIV-1 infected patients were characterized by tremor, dyskinesia, disturbance of intellect, forgetfulness, apathy, poor concentration as well as sequential processing, the symptoms similar to those found in subcortical dementia of Parkinson's disease [9, 10]. Subsequent findings that in the CNS, high concentrations of virus was present in the hippocampus and basal ganglia, the areas that are rich in dopaminergic activity, led to the concept that deficits in dopamine might be associated with the neurological disorders, cognitive impairment and behavioral changes [11-14].

Dopaminergic neuronal activity in these same regions have been associated with the regulation of various cognitive functions, including verbal functions, perceptual motor functions and motor planning, cognitive flexibility, concentration, attention and speed of information processing, learning, working memory, and executive functions [15, 16].

Dopamine is synthesized in the CNS dopaminergic neurons (localized in the substantia nigra) and in the peripheral systems (nerve terminals and the adrenal glands) from circulating L-tyrosine, the essential amino acid obtained from dietary sources. Tyrosine is converted to L-3, 4-dihydroxyphenylalanine (L-DOPA) by the rate-limiting enzyme, L-tyrosine hydroxylase, and in turn L-DOPA is converted to dihydroxyphenylethylamine (dopamine) by the enzyme L-dopa decarboxylase. Dopamine is metabolized to its major metabolite, homovanilic acid (HVA) by the sequential activity of two enzymes, catechol-o-methyltransferase COMT) and monoamine oxidase (MAO). Neuronal projections emanating from the substantia nigra (SN) transport dopamine into the striatum, hippocampus, and other regions forming fronto-striatal circuitries with afferent and efferent terminals relayed from basal ganglia to frontal cortex and other regions as shown in figure 1. As earlier findings suggested that high concentration of HIV-1 was present in the brain regions [14] which have been known to be rich in dopamine. Since deficits in neurological and neurocognitive functions found in HIV-1 infected patients were similar to those found in patients with Parkinson's disease (PD), such as bradykinesia, postural instability and their striking sensitivity to dopamine blocking agents, such as metoclopropamide, or chlorpromazine [17], it was conceptualized that central dopamine deficiency may occur in HIV-1 infection. The support for this concept was provided by the studies carried out by our group and by others showing a decrease in the levels of dopamine, as well as HVA, in the CSF of patients who were infected with HIV-1 [18,11]. Since, the relationship between the decreased levels of dopamine in the CNS and cognitive dysfunctions caused by HIV-1 are not clearly understood, we investigated the status of dopamine in different regions of post mortem brains of HIV-1 infected patients who were assessed for cognitive performance during life.

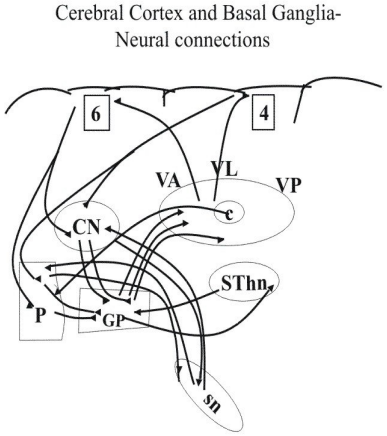

Fig. 1: CN=Caudate nucleus, P=Putamen, GP=Globus pallidus, sn= substantia nigra, VA=Anterior part of the ventral nucleus, VL=Lateral part of the ventral nucleus, VP=Posterior part of the ventral nucleus, SThn=Subthalamic nuclei

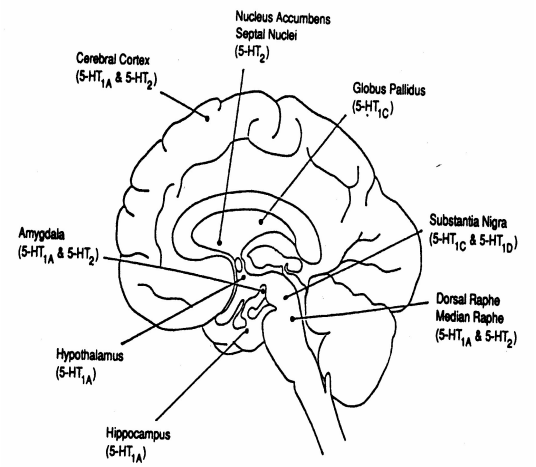

Fig. 2: The diagrammatic presentation of the brain showing various regions, including raphe nuclei where serotonin (5-HT) is synthesized. 5-HT is transported to different brain regions where it binds to various types of 5-HT receptors which regulate serotonergic activity

Furthermore, among HIV-1 infected individuals mental health and behavioral problems are widely prevalent and are characterized by disruption of mood [19,20], depressive symptoms, major depression [21], eating disorders [22], and aggressive and suicidal behaviors [23]. Although a number of social factors (social stigma, financial burdens, and the recognition that it is a fatal disease), have been associated with the mental disturbances in HIV-1 infected patients [24], investigations on the relationship between the behavioral problems and deficits in serotonin functions caused by HIV-1 infection, have received limited attention. Since serotonin deficiency has been implicated in a number behavioral and psychiatric problems such as depression, aggression, suicide, and eating disorders [25], and since HIV-1 infected patients experience these behavioral problems, we hypothesized that serotonin deficiency may occur in HIV-1 infected patients.

Serotonin is synthesized in the enterochromaffin cells of the peripheral systems and in the mid brain raphae nuclei from the dietary essential aminoacid, L-tryptophan which is converted by the rate limiting enzyme, tryptophan hydroxylase to L-5-hydroxytryptophan (5-HTP), which in turn is converted by the enzyme, 5-HTP-decarboxylase to 5-hydroxy-tryptamine (5-HT, serotonin). 5-HT is further metabolized to 5-HIAA by the mitochondrial enzyme monoamine oxidase (MAO). The neuronal projections emanating from raphe nuclei transport 5-HT to different brain regions by the mechanism similar to that of dopamine transport from substantia nigra to different regions. The monoamine neurotransmitters, DA and 5-HT are also known to interact and regulate the activity of each other in various brain regions by binding to their specific type of receptors (figure 2) found in many brain regions [26]. However, investigations regarding the status of these monoamine neurotransmitters in the CNS of HIV-1 infected individuals remain scarce. Although, as mentioned above, HIV-1-associated neuropathogenesis leading to cognitive deficits, and motor disorders, and dementia [27- 29, 8, 20], as well as mental health problems have been widely described in the majority of the HIV-1 infected individuals with AIDS [20], the association of the adverse affect of HIV-1 infection in the brain regions associated with the central dopaminergic and serotonergic activity is not fully understood. In this paper we report the status of CNS monoamine neurotranmitters in some of the brain regions affected by HIV-1 infection. Since we anticipated that the concentration of dopamine and HVA in the brain regions of HIV-1 infected individuals may be decreased substantially, we used highly sensitive CoulArray HPLC-ECD system to determine their concentration in small quantity of available tissues by the method described earlier [30]. We also quantified HIV-1 viral load in the same brain regions of cases using real time reverse transcriptase chain reaction (real time RT-PCR).

MATERIALS AND METHODS

Human post mortem brain tissues used for measurement of neurotransmitters, dopamine and its metabolite, HVA, were procured from the four NIH supported National NeuroAIDS Tissue Consortium (NNTC) centers. The NNTC centers obtained the informed consent of the individuals and their family, and approval of institutional review board for human ethics prior to enrolment of individuals for evaluation of their health status every six months during life, including physical, psychological, mental health, and neurocognitive functions, as well as for donation of organs and body fluids as gift after their death for research investigations related to HIV/AIDS (Anatomical Gift Act, 1990). The individuals excluded from the study were those who had neuropsychiatric illness such as schizophrenia, opportunistic infections of the CNS, if they were on antipsychotic medications, had history of dependency on stimulant drugs, had CNS cancer, and complications due to severe head injury with loss of consciousness for more than 30 minutes. The brain tissues of HIV-1+ and HIV- negative cases

were collected at the NNTC centers at the time of autopsy performed within 24 hours of an individual's death, and according to the guidelines established by the consortium advisory for harvesting the postmortem tissues. The tissues used for this study were flash frozen in liquid nitrogen and preserved at –80°C. The Institutional Review Board of the University of Miami approved the study presented in this paper. The overnight shipping and handling of tissues in dry ice from the NNTC centers to the site of these investigations was carried out according to the criteria established by the NIH and US Federal Regulations (49 CFR 172 subpart H).

The HIV-1+ individuals included in this study had been infected with HIV-1 for variable number of years (<1-23 years), and died between 1998-2005, and a majority of them had received antiretroviral therapy. The HIV-negative individuals had also died during the same span of time as HIV-1+ individuals, but their death was due to different causes. In this report, the brain regions included are the frontal cortex (FC), basal ganglia (BG), substantia nigra (SN) of both HIV-1+ and HIV-negative individuals. Due to limited availability of all regions from each individual, investigations were carried out in the brain regions that were available.

The status of 5-hydroxytryptamine was assessed in an earlier study in CSF of HIV-1 infected patients during life [13]. This study was carried out in a separate group of asymptomatic HIV-1 infected individuals who were among those enrolled in a longitudinal prospective study of neurological complications of HIV-1 infection. The study was approved by the Institutional Review Board (IRB) of the University of Miami School of Medicine. Samples of CSF were collected by lumbar puncture while the subject was in a lateral recumbent position. After centrifugation of CSF for 10 min at 4°C to eliminate debris and cells, one milliliter aliquots were stored in amber color tubes at –80° C until analyzed by HPLC-ECD.

Extraction and Quantification of Dopamine and HVA : Brain tissues were thawed, weighed and homogenized in buffer (1:10), and DA and HVA were extracted and quantified separately as describe earlier using highly sensitive CoulArray HPLC-ECD system [30]. Briefly, the tissue homogenates were treated with perchloric acid (PCA) to precipitate proteins and after centrifugation at 13000 rpm and at 4°C, the supernatant was spiked with internal standard, dihydroxybenzylamine (DHBA), and the mixture was treated with activated alumina for adsorption of catecholamines (CATs) including dopamine. Alumina was washed twice with diluted (1:100), 2M TRIS-HCL, pH 8.7, and CATs were eluted with 200 µl of 0.1M acetic acid, and an aliquot, 50 µl of the elute was injected in to catecholamine column (reverse phase, 5µ, C18), and separation carried out isocratically with the mobile phase at a flow rate of 0.7 ml/min. Peaks of DA and internal standard were identified by their retention time and concentration was determined by the ratio of the peak area of the sample to that of the known concentration of internal standard. The values of dopamine were expressed as pg/g tissue

Extraction of HVA from tissues involved precipitation of proteins with PCA and extraction with diethylether, and isoproterinol was used as an internal standard. Quantification of HVA was carried out with the procedure similar to that of DA using CoulArray HPLC-ECD system as previously describe [30]. HIV-1 RNA load was determined in the same brain regions using real time RT-PCR with sensitivity of <5 copies of HIV-1 RNA, , using primers and probes from the long terminal region (LTR) of HIV-1 genome by the procedure described in detail elsewhere [31].

Extraction and measurement of 5-HT and 5-HIAA in CSF have been described earlier. Briefly 5-HT and 5-HIAA were extracted with perchloric acid (PCA) and quantified simultaneously using HPLC-ECD, with N-methyl-5-hydroxytryptamine as an internal standard [32].

RESULTS

The studies described in this paper demonstrate the preliminary findings of the affect of HIV-1 on the status of brain monoamine neurotransmitter systems. The data were analyzed by using Student's t test, when applicable, to examine the significance of difference in the values of neurotransmitters, between HIV-1+ and HIV-1- individuals. We found wide variation in the values of dopamine and HVA, as well as HIV-1 viral load in the brain regions and the data are given as the range of values as obtained from the brain tissues of HIV-1+ and HIV-1- cases.

The demographic characteristics of HIV-1+ and HIV-negative cases is presented in table 1. The HIV-1+ cases had been infected with the virus for variable number of years, from less than 1 year to 23 years, and 86 % of them were men and the rest, 14 % were women, belonging to different ethnic groups. Their age at death was similar to that of HIV- cases. The majority of HIV-1 infected individuals had received antiretroviral therapy during life.

Table 1: Comprehensive Demography of HIV-1+ and HIV-1- cases

HIV-1+ Cases	Specific Characteristics	HIV- cases
Year Infected	1978-2001	Not applicable
# years of Infection	<1-23	Not applicable
Age at Death (years)	31-58	31-55
Year of death	1998-2005	1998-2005
Gender	86 % M and 14 % F	54.5 % M, 45.4 % F
Ethnicity	76 % Caucas, 10.5 % Hisp, 18.2 % AA, 27.3 % Native Am	82 % Cauc, 18 % AA
Antiretroviral therapy	yes, 86.8 %, no, 13.2 %	Not applicable

Dopamine (DA) concentration (pg/g Tissue) was found to have a wide range in the brain regions of HIV-1+ individuals, and even within the specific region, the range of DA concentration (pg/g tissue) was found to be substantially decreased (0.84-9450) when compared with the range of values in the same regions of HIV-negative individuals (12.1-77500), except in FC where DA levels were not reduced in HIV-1 + cases (The values of DA ranged between 0.84- 6086.0, in HIV-1+ individuals, compared to 24.3-923,0 in FC of HIV-negative brains,). Similar trend was observed in the values of HVA (ng/g tissue), showing decreased concentration in the same brain regions of HIV-1 + cases, (1.09-2571.6 in HIV-1+, compared to 4.52-2703 in HIV- cases). The HIV-1 load (VL, $\log_{10}$ copies/g tissue) in the same brain regions were also found to be widely variable (VL $\log_{10}$ range = 0.74 – 6.66, the highest viral RNA concentration was found in the caudate nucleus of the basal ganglia. Moreover, viral RNA was not detectable in the brain regions of a large number of HIV-1 infected cases (33.3 %-64 %) and none of the brain regions of HIV-1- cases had detectable VL.

With respect to 5-HT and 5-HIAA (ng/ml) in CSF as described earlier, the levels were significantly decreased in HIV-1 infected patients (HIV-1+, 5-HT= 0.23±0.31) compared to that in the control subjects (HIV-, 5-HT= 3.53±3.80; p< 0.000), but there was no difference in the values of 5-HIAA between the two groups (HIV-1+, 5-HIAA=14.3 ± 4.91 vs HIV-1-, 5-HIAA= 15.6±.7.3, p= NS).

DISCUSSION

This study demonstrates that dopamine concentration in the brain regions of HIV-1 infected persons is decreased. Although, wide variability was found in dopamine levels in the same brain regions of different individuals, a substantial decrease in DA levels was found in the basal ganglia and substantia nigra of HIV-1 infected cases. Similar pattern was found in the values of HVA, however, due to wide variation in the values in each region, the significance of difference in the levels of both DA and HVA between HIV-1+ and HIV- could not be evaluated. The relationship between CNS regional dopamine and viral load, as well as that of assessment of neurocognitive functions and the role of antiretroviral therapy during life, will be described elsewhere.

Although, the exact mechanisms involved in the degenerative process of dopaminergic neurons are not clearly understood, and whether the decrease in the levels of DA and HVA observed in the brain regions in this study is due to a decrease in the synthesis or increased metabolism of DA to HVA, or it is a result of combination of both processes, however, the findings of decreased DA in the CNS have been consistent in different studies, that used CSF (11) and the caudate nucleus [33] of HIV-1+ infected individuals. It is well recognized that the neurons themselves are not directly infected by the virus [34, 35], since the neurons do not express CD4 receptor molecules [36], the neuronal damage is probably caused by the neurotoxic viral proteins, such as gp120, and Tat, as well as other cytotoxic products produced by the host cells in response to HIV-1 infection [37-39]. The role of viral protein, Tat, in the reduction of dopamine synthesis has been elucidated by the in vitro studies carried out in cell culture using PC12 dopaminergic cell lines, and it was found that Tat inhibited the expression of tyrosine hydroxylase [40], the key enzyme required for dopamine synthesis from the dietary essential aminoacid, tyrosine. Also, the evidence from a few preclinical and clinical findings suggests that the extrapyramidal motor dysfunctions found in HIV-1 infected patients are associated with the dopaminergic neuronal injury in the substantia nigra, similar to that found in patients with PD [17].

Furthermore, the drugs of abuse such as cocaine, amphetamines and methamphetamine have been known to produce the euphoric effect by increasing many folds (100-1000 fold) the synaptic dopamine levels and ultimately depleting the neuronal dopamine and

resulting in the generalized CNS dopamine deficiency. Recent studies show that the decrease in the levels of dopamine in CSF is exacerbated in HIV-1 infected patients who are also methamphetamine users and dependent [41].

Regarding the status of serotonin in CSF of HIV-1 infected patients, it was found that while concentration of 5-HT was significantly decreased in the HIV-1 infected patients [13], there was no change in the concentration of 5-HIAA, the major metabolite of 5-HT. However, the levels of CSF 5-HT in HIV-1 infected patients did not relate with depression evaluated with Beck's depression scale during life. Recent reports suggest that one of the important contributing factors for serotonin deficiency in HIV-1 infection may be the disturbed metabolism of tryptophan and its decreased availability for serotonin synthesis, since HIV-1 infected macrophages secrete large amounts of cytokines which direct tryptophan to an alternate metabolic pathway, the L-tryptophan - kynurenine pathway, resulting in the production of high concentration of the neurotoxic products such as quinolininc acid [42-44, 20].

Moreover, HIV-1 infection related disturbances have also been reported in the activity of other neurotransmitters associated with autonomic activity, as well as HPA axis function [45, 46].

Summary: This study demonstrates that HIV-1 infection adversely affects the central monoamine neurotransmitter systems, dopamine and serotonin. The wide variability found in the levels of dopamine in different brain regions may be due to the difference in the sensitivity of these regions to neurotoxic produts secreted by the host cells in the brain in response to HIV-1 infection. Moreover, wide variability found in HIV-1 RNA load in different brain regions may also contribute to difference in the regional dopaminergic damage.

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