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Short Communication

Suppression of human immunodeficiency virus replication in human brain tissue by nucleoside reverse transcriptase inhibitors

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Human immunodeficiency virus (HIV) infection of the brain is associated pathologically with neuronal damage and loss. Clinically cognitive impairments can develop, which in some can be improved by highly active antiretroviral therapy (HAART), whereas in others, the infection persists despite treatment. The efficacy of antiretrovirals to treat cognitive impairments may be related to their ability to suppress viral replication in the brain and also to prevent neurodegeneration. To investigate this question, the authors assessed the ability of stavudine (300 nM), zidovudine (2 nM), and abacavir (300 nM) to suppress viral replication in human brain tissue aggregates infected with HIV-1 SF162. Aggregates were cultured for 4 weeks and exposed to nucleoside reverse transcriptase inhibitors (NRTIs) either 24 h prior, simultaneously, or 24 h post infection. Viral replication was assessed by p24 enzyme-linked immunosorbent assay (ELISA) in culture medium. The authors observed a statistically significant reduction in the rate of viral replication for stavudine added 24 h prior to infection univariate analysis of variance ([UANOVA], $t = 2.55$, $df = 17$, $P = .021$). Decreased viral replication observed with zidovudine and abacavir was not statistically significant. *Journal of NeuroVirology* (2004) **10,** 136–139.

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In the brain, human immunodeficiency virus (HIV) is associated with inflammatory disorders, e.g., HIV encephalitis, synaptic and dendritic damage, and neuronal loss (Everall *et al*, 1991; Masliah *et al*, 1997). HIV viral load in the brain, together with neuronal damage and loss, correlates with the presence of cognitive impairments (Everall *et al*, 1991; Masliah *et al*, 1997). This indicates that damage caused by HIV to the brain that leads to cognitive impairment and dementia results from increasing viral burden.

Highly active antiretroviral therapy (HAART) suppresses systemic viral replication, and a number of studies have reported improvements in cognitive deficits (Chang *et al*, 1999). However, not all therapies are as efficacious, because cognitive impairments can persist while on treatment (Tozzi *et al*, 1999). Therefore, it is important to obtain data on the efficacy of various antiretroviral agents to aid clinicians in treatment decisions, especially because early neuronal injury, such as occurs in mild cognitive deficits (Masliah *et al*, 1997; Everall *et al*, 1999), may be reversible. We decided to investigate the ability of three nucleoside reverse transcriptase inhibitors (NRTIs) to suppress viral replication over time. We assessed stavudine (300 nM), zidovudine (2 nM), and abacavir (300 nM) at drug concentrations found in the cerebrospinal fluid (CSF) and within the IC_{50} range. To accomplish this, we used the human fetal brain aggregate model composed of neurons, astrocytes, microglia/macrophages, and oligodendrocytes (Trillo-Pazos *et al*, 2003). Briefly, as

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described previously by Trillo-Pazos *et al* (2003), human fetal brain tissue was cultured on 0.75% Noble agar-coated plates in Dulbecco's modified Eagle's serum and 5% human serum for 4 weeks. HIV-1 SF162 viral stocks were grown in peripheral blood monocyte cells with interleukin-2 and phytohemagglutinin. Aggregates were infected overnight with HIV-1 SF162 at 1:10 dilution of stock solution (equivalent to 1000 pg/ml p24) and on the following day, they were washed thoroughly to remove of any excess virus (Kandanearatchi *et al*, 2002). Stavudine, zidovudine, or abacavir was added either 24 h prior to, simultaneously with, or 24 h following infection. Three aggregates were infected but left untreated, and a further three aggregates that were not HIV infected served as controls. In each condition, there were three aggregates and each experiment was carried out three times. Viral replication was assessed by the presence of p24 immunopositivity in the culture medium by enzyme-linked immunosorbent assay (ELISA; Coulter, UK) over 12 days. An increase in p24 levels was observed by day 3, which then gradually decreased to day 12. For each aggregate, the rate of increase in viral replication between days 0 and 3 was calculated by linear regression. Similarly, the rate of decrease from days 3 to 12 was estimated by linear regression. The replication rates under each of the NRTI treatments were compared to that of infected aggregates when left untreated using contrast tests in an analysis of variance (ANOVA) model.

As excess virus was washed off thoroughly following infection, no p24 was detected on day 0. In infected, untreated aggregates, peak p24 levels in the supernatant on day 3 reached approximately 60 pg/ml (Figure 1). Stavudine, when added 24 h prior to infection, significantly attenuated the p24 peak by 50% to 30 pg/ml, compared to the peak in untreated infected aggregates (Figure 1), and resulted in a significant reduction in the rate of increase in viral replication (Table 1; $t = 2.55$, $df = 17$, $P = .021$). Again, reductions in the rate of decrease were only significant for pretreatment with stavudine 24 h prior to infection (Table 1; $t = 2.42$, $df = 17$, $P = .027$). However, when stavudine was added simultaneously or 24 h following infection, the rise and decline of p24 levels were not statistically significantly different compared to the rates of increase and decrease in untreated infected aggregates (Table 1). Also, neither zidovudine nor abacavir had any significant impact on the rates of increase or decrease in p24 levels, even when added 24 h prior to infection with HIV-1 SF162. In this situation, peak p24 levels were over 40 pg/ml when treated with zidovudine or abacavir, whereas they remained less than 30 pg/ml in the stavudine-treated aggregates. Although reports on drug concentrations reaching human brain tissue are scarce, a clinical study reported CSF concentrations of stavudine achieving higher levels in comparison to zidovudine (Foudraine *et al*, 1998). Furthermore, studies

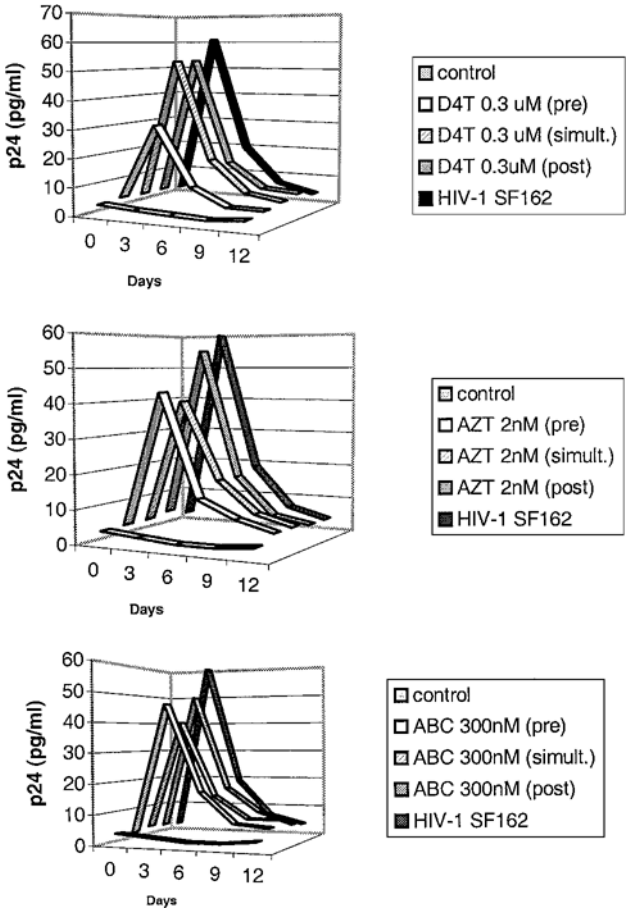

Figure 1 p24 levels for aggregates treated with stavudine (D4T) (i); zidovudine (AZT) (ii); and abacavir (ABC) (iii) either 24 h prior to, simultaneously, or 24 h following infection between days 0 and 12.

using animals, however, do indicate that stavudine does reach brain tissue (Yang *et al*, 1997).

Most of the reported studies on the efficacy of antiretroviral treatment of HIV have been on zidovudine because it was the first antiretroviral agent used in the treatment of HIV disease, whereas there are fewer studies on the efficacy of stavudine or abacavir in the brain. Moreover, these investigations on stavudine or abacavir in the central nervous system have been performed on either whole animal models or on *in vitro* microglia monolayer cultures (Limoges *et al*, 2000; Hong *et al*, 2000). Therefore, we believe that it was important to observe these effects on primary human brain tissue in a model that resembles the *in vivo* situation as closely as possible, hence our use of the human brain aggregate model. The important observation of the current study is that stavudine, administered prior to infection of the human brain aggregates, significantly reduced viral replication. This finding is important for two reasons. Firstly, the rising brain viral burden is associated with increasing neuronal damage and death, as well as clinically with worsening cognitive impairments

Table 1 Comparison of rate of viral replication (measured in pg/ml/day p24 ELISA) between NRTI-treated HIV-infected aggregates and untreated aggregates

	Difference in rate of increase (days 0–3) in viral replication (per day) [HIVSF162 minus NRTI-treated]				*Difference in rate of decrease (days 3–12) in viral replication (per day) [HIVSF162 minus NRTI-treated]*			
NRTI	*Rate of change (pg/ml/day)*	*95% CI*	*Contrast t test (df = 17)*	P *value*	*Rate of change (pg/ml/day)*	*95% CI*	*Contrast t test (df = 17)*	P *value*
Stavudine 300 nM								
Pre	10.5	[1.8, 19.2]	2.55	.021	3.1	[0.4, 5.9]	2.42	.027
Simultaneous	2.9	[−5.8, 11.6]	0.71	.49	0.9	[−1.8, 3.7]	0.73	.48
Post	2.6	[−6.1, 11.3]	0.63	.54	1	[−1.7, 3.7]	0.78	.45
Zidovudine 2 nM								
Pre	6.3	[−2.4, 15]	1.52	.15	2.1	[−0.7, 4.8]	1.61	.13
Simultaneous	7.1	[−1.6, 15.8]	1.72	.1	2.2	[−0.5, 4.9]	1.69	.11
Post	2.4	[−6.3, 11.1]	0.59	.56	0.6	[−2.2, 3.3]	0.45	.66
Abacavir 300 nM								
Pre	5.1	[−4.7, 14.8]	1.1	.29	1.7	[−1.3, 4.8]	1.19	.25
Simultaneous	7	[−2.7, 16.8]	1.53	.15	2.5	[−0.5, 5.6]	1.74	.1
Post	4.2	[−5.5, 13.9]	0.92	.37	1.4	[−1.7, 4.5]	0.97	.35

Note. Viral replication as indicated by the level of p24 immunopositivity in culture media is shown. For each aggregate, the rate of replication between days 0 to 3 and 3 to 12 were assessed by linear regression, then the regression coefficients were compared between the various conditions and the infected group (*P* values).

(Masliah *et al*, 1997; Everall *et al*, 1999; Ellis *et al*, 1997). Thus the ability to suppress viral replication will prevent or minimize HIV neurodegenerative disorder. Our finding of the ability of stavudine to attenuate the rate of viral replication, together with our demonstration of its ability to prevent neuronal loss in this model (Kandanearatchi *et al*, 2002), supports the notion that it maybe useful clinically in preventing or treating HIV-related cognitive impairments. This leads to our second point that the superior efficacy of stavudine was apparent when added prior to infection with HIV. Also, asymptomatically infected individuals have low or usually undetectable levels of provirus in the brain (Bell *et al*, 1998); our results would indicate that stavudine administered prophylactically during this period may offer better protection in suppressing viral replication and preventing neuronal loss and HIV neurocognitive disorder. Finally, although blood-brain barrier penetration was not assessed in this study, the drug concentrations used are those expected to be achieved in the brain, especially as the other drugs of a particular combination regime may boost plasma levels and therefore enhance brain concentrations (Thomas and Segal, 1998; Takasawa *et al*, 1997).

References

Bell JE, Brettle RP, Chiswick A, Simmonds P (1998). HIV encephalitis, proviral load and dementia in drug users and homosexuals with AIDS—effect of neocortical involvement. *Brain* **121:** 2043–2052.

Chang L, Ernst T, Leonido-Yee M, Whitt M, Speck O, Walot I, Miller EN (1999). Highly active antiretroviral therapy reverses brain metabolite abnormalities in mild HIV dementia. *Neurology* **53:** 782–789.

Ellis RJ, Hsia K, Spector SA, Nelson JA, Heaton RK, Wallace MR, Abramson I, Atkinson JH, Grant I, McCutchan JA, the HNRC Group (1997). Cerebrospinal fluid human immunodeficiency virus type 1 RNA levels are elevated in neurocognitively impaired individuals with acquired immunodeficiency syndrome. *Ann Neurol* **42:** 679–688.

Everall IP, Heaton RK, Marcotte TD, Ellis RJ, McCutchan JA, Grant I, Mallory M, Masliah E, HNRC Group (1999). Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. *Brain Pathol* **9:** 209–217.

Everall IP, Luthert PJ, Lantos PL (1991). Neuronal loss in the frontal cortex in HIV infection. *Lancet* **337:** 1119–1121.

Foudraine NA, Hoetelmans RMW, Lange JMA, de Wolf F, van Benthem BHB, Maas JJ, Keet IPM, Portegies P (1998). Cerebrospinal fluid HIV-1 RNA and drug concentrations after treatment with lamivudine plus zidovudine and stavudine. *Lancet* **351:** 1547–1551.

Hong M, Schlichter L, Bendayan R (2000). A Na(+)-dependent nucleoside transporter in microglia. *J Pharmacol Exp Ther* **292:** 366–374.

Kandanearatchi A, Zuckerman M, Smith M, Vyakarnam A, Everall IP (2002). Granulocyte-macrophage colony-stimulating factor enhances viral load in human brain tissue: amelioration with stavudine. *AIDS* **16:** 413–420.

Limoges J, Persidsky Y, Poluektova L, Rasmussen J, Ratanasuwan W, Zelivyanskaya M, McCernon DR, Lanier ER, Gendelman HE (2000). Evaluation of antiretroviral drug efficacy for HIV-1 encephalitis in SCID mice. *Neurology* **54:** 379–389.

Masliah E, Heaton RK, Marcotte TD, Ellis RJ, wiley CA, Mallory M, achim CL, McCutchan A, Nelson JA, Atkinson JH, Grant I, HNRC Group (1997). Dendritic injury is a pathological substrate for HIV related cognitive disorders. *Ann Neurol* **42:** 963–972.

Takasawa K, Terasaki T, Suzuki H, Ooie T, Sugiyama Y (1997). Distributed model analysis of 3′-azido-3′-deoxythymidine and 2′,3′-deoxyinosine distribution in brain tissue and cerebrospinal fluid. *J Pharmacol Exp Ther* **282:** 1509–1517.

Thomas SA, Segal MB (1998). The transport of the anti-HIV drug, 2′,3′-didehydro-3′-deoxythymidine (D4T), across the blood-brain and blood-cerebrospinal fluid barriers. *Br J Pharmacol* **125:** 49–54.

Trillo-Pazos G, Kandanearatchi A, Eyeson J, King D, Vyakarnam A, Everall I (2003). HIV-1 causes neuronal loss and damage in human brain balls: a human 3-D model to investigate the cellular brain of HIV associate dementia. *Neuropathol Appl Neurobiol* In press.

Tozzi V, Balestra P, Galgani S (1999). Positive and sustained effects of highly active antiretroviral therapy on HIV-1 associated neurocognitive impairment. *AIDS* **13:** 1889–1897.

Yang Z, Brundage RC, Barbhiaiya RH, Sawchuk RJ (1997). Microdialysis studies of the distribution of stavudine into the central nervous system in the freely moving rat. *Pharmacol Res* **14:** 865–872.