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Clinical Science Workshop 1

# CSF/peripheral nervous system

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### Viral load markers and neurological status: a report from the North-East Dementia Cohort

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To investigate the relationship between plasma and CSF HIV RNA levels, immune activation markers, and neurological status in a cohort with advanced HIV infection. HIV-associated dementia (HAD) occurs in 15% of patients with HIV/AIDS. Previous work has shown that levels of CSF HIV RNA and selected immune activation markers correlate with the severity of neurological disease. Methods: 362 subjects with CD4 < 200, or cognitive symptoms and CD4 <300 were enrolled in the NEAD cohort. HAD and MCMD are diagnosed using the AAN criteria, applied through a computerized algorithm (Marder, 1996). CSF HIV RNA is performed using NucliSens QT (LOD 50 copies/ml). MCP-1, MMP-2 and TNFa are assayed with ELISA kits. Results: At baseline (1998–9) the cohort demographics were: mean age 42 ± 7 years, 65% black, and 30% female. Mean logCD4 count was 4.6 ± 1.0, HAART was currently used in 71%, and 62% had an AIDS-defining illness. The neurological categorization at baseline was: 30% Normal (n = 108); 34% MCMD (n = 121); and 36% HAD (n = 128). The degree of HAD was mild in the majority of subjects. Mean plasma HIV RNA was 3.9 ± 1.3, and CSF HIV RNA was 2.6 ± 0.9, with no differences among the groups, even after adjustments for enrollment center, baseline CD4, and antiretroviral therapy. CSF HIV RNA was undetectable in 52%, 46%, and 39% respectively (p = NS). For the immune activation markers, no significant differences were found among the different groups. Conclusion: In contrast to earlier, smaller studies which have shown significant correlations between these CSF markers and neurological status, in this "enriched" cohort with advanced HIV/AIDS in the era of HAART no such relationship was found at baseline. The difference was not explicable by antiretroviral usage, demographics, or plasma virological control. Neurological categorization was identical using other validated strategies. Our observations suggest that with widespread HAART usage, HIV RNA and immune activation markers may fail to discriminate milder degrees of HIV-associated neurocognitive impairment in advanced HIV/AIDS. Longitudinal studies will define the predictive utility of these markers for subsequent neurological progression.

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### HIV-1 pol analysis of paired cerebrospinal fluid and plasma specimens: correlation between pairwise nucleotide diversity and HIV-associated dementia

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Background and objective: Viral load studies of paired cerebrospinal fluid (CSF) and plasma specimens indicate that HIV replication can be variably segregated in these two compartments. Objective of this study was to evaluate the extent of variation between CSF and plasma HIV-1 pol sequences and its association with HIV-induced CNS disease.

Methods: Paired CSF and plasma specimens from 48 neurologically symptomatic HIV-infected patients, including 23 antiretroviral naive, 19 RTI-experienced and 6 RTI and PI-experienced patients, were retrospectively examined. Viral load was assessed by RT-PCR (Roche Amplicor Monitor) and reverse transcriptase (RT) and protease sequences were obtained by direct sequencing (Applied Biosystems 7700). Sequencing data were analysed for presence of resistance mutations; nucleotide distance between CSF and plasma sequences was calculated by using the Kimura 2-parameter model.

Results: RT resistance mutations were found in CSF and/or plasma of 26 patients, respectively, including three antiretroviral naive patients. A different CSF/plasma pattern was observed in 10/48 patients (22%), with mutations present in CSF but not in plasma in four (8%). Protease resistance mutations were observed in 24/29 patients, in all but one case consisting of accessory mutations at codons 10, 36, 63 or 77. No correlation was found between the CSF/plasma nucleotide distance and type of CNS disease, CSF cell counts, CSF or plasma viral load, CSF to plasma VL ratio or duration of HIV infection. Neither was any of these factors associated with the presence of a different CSF/plasma resistance pattern. However, when only the antiretroviral naive patients were analysed, higher CSF/plasma distance values were observed in patients with dementia than in those without (RT: median 0.0192 vs. 0.0110, p = 0.008; protease: median 0.0384 vs. 0.0205, p = 0.019).

Conclusions: In patients with HIV dementia, and in absence of antiretroviral therapy, relatively distant pol

sequences may be observed between CSF and plasma, possibly supporting an autonomous HIV replication in the CNS.

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## Immunophilin ligand, FK506 is neuroprotective in *in vitro* models of HIV-associated peripheral neuropathies

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Background and Rationale: HIV-associated painful sensory neuropathies (DSP: Distal Sensory Polyneuropathy and ATN: Antiretroviral Toxic Neuropathy) are the most common neurological complications of HIV infection in this era of HAART regimens. Recently, we developed *in vitro* models of DSP and ATN using gp120 and ddC, respectively, on primary dorsal ganglion sensory neurons.

Methods: Primary DRG neurons from embryonic day E14–16 animals were harvested and co-cultured with Schwann cells. After establishing neurites, varying doses of FK506 or vehicle were added to the wells with ddC or gp120. At the end of the incubation, cells were fixed and stained with a neuronal marker (anti-ßIII tubulin antibody). Neurotoxicity was assayed as an inhibition of neurotic outgrowth and degeneration of established neuritic processes. We used JC-1 fluorescence, and TUNEL staining to study the effects of these compounds on the mitochondria and apoptotic pathways.

Results: ddC in the low $\mu$M and gp120 in the nM range caused a dose-dependent neurotoxicity on primary DRG sensory neurons. This neurotoxicity was preventable by nM doses of FK056, but not cyclosporin-A. FK506 protected the DRG neurons against loss of neuritic numbers and reduction in total neuritic length. Furthermore, FK506 also prevented the mitochondrial toxicity seen with exposure to ddC. ddC caused cell death in a susceptible subpopulation of DRG neurons and this was prevented by addition of FK506 to the culture medium at the time of exposure to ddC.

Discussion: We used *in vitro* models of HIV-associated sensory neuropathies and showed that FK506 is neuroprotective against the toxicity of ddC and gp120. Since FK506 is an immunosuppressive immunophilin ligand, its use in HIV patients will be limited. However, nonimmunosuppressive analogues of FK506 are being developed and hold a promise to prevent painful neuropathies associated with the HIV infection and its treatment.

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## Morphological abnormalities and mitochondrial dysfunction induced by NRTI in human dorsal root ganglia neurons

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Objective: To determine the effects of nucleoside reverse transcriptase inhibitors (NTRI) on human dorsal root ganglia (DRG).

Background: Surprisingly, the drugs used to treat HIV infection (NRTI) can cause a painful distal sensory neuropathy clinically indistinguishable from that caused by HIV infection itself. Hence understanding the pathogenesis of this neuropathy is critical for development of new modes of therapy.

Design/methods: Organotypic human DRG cultures were establish from 58–72 days gestational age fetuses. After two weeks in culture, they were exposed to NRTI (ddC, ddI, d4T, 3TC or AZT) (1–20 ug/ml) for another two weeks. The cultures were analyzed by light and electron microscopy, immunostaining for neuronal markers and quantitative morphological analysis. Changes in intracellular calcium and mitochondrial function by JC-1 assay were monitored in select neuronal cultures.

Results: The earliest changes noted were shortening and loss of neurites followed by cell death in select neuronal populations. Ultrastructural abnormalities included loss of cristae in mitochondria, clustering of neurofilaments and microtubules, accumulation of glycogen like particles, and an increase in ribosomes. Morphological changes induced by ddC, ddI and d4T were indistinguishable. 3TC treatment showed striking accumulation of lysosomes where as AZT showed no toxicity. The neurotoxicity induced by NRTI was observed as follows: ddC > ddI > d4T > 3TC > AZT with significant shortening and reduction in number of neurites. ddC and ddI also caused significant decreases in mitochondrial potential and increases in intracellular calcium. When ddC was applied following exposure to gp120, select neuronal populations showed synergistic increases in intracellular calcium from which they failed to recover.

Conclusions: NRTI cause select but synergistic neurotoxicity in human DRGs associated with disruption of neurites and mitochondrial toxicity. 3TC may have a unique mechanism of neurotoxicity. Our studies support the use of AZT as a NRTI of choice in HIV infected patients at risk of peripheral neuropathy.

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