

Sensory Neuropathy in Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients: Protease Inhibitor–Mediated Neurotoxicity

Jacqueline A. Pettersen, MD, MSc,¹ Gareth Jones, PhD,¹ Catherine Worthington, PhD,² Hartmut B. Krentz, PhD,³ Oliver T. Keppler, MD, PhD,⁴ Ahmet Hoke, MD, PhD,^{1,5} M. John Gill, MB,^{6,7} and Christopher Power, MD^{1,7,8}

Objective: Human immunodeficiency virus–associated sensory neuropathy (HIV-SN) is a common and disabling disorder, often associated with antiretroviral therapy (ART) use. We investigated the clinical features and associated pathogenic determinants of HIV-SN in a neurological cohort of HIV-infected patients, together with a novel model of HIV-SN.

Methods: HIV-infected patients with neurological disease were investigated in terms of clinical and laboratory aspects together with ART exposure focusing on symptomatic HIV-SN. Rat-derived dorsal root ganglion (DRG) cultures, transgenic for human CD4 and CCR5 treated with ARTs or HIV infected, or both, were studied with respect to quantitative neuronal injury.

Results: Among 221 patients assessed from 1998 to 2004, 120 had no sensory neuropathy, whereas 101 displayed HIV-SN, including 64 with distal sensory neuropathy and 37 with antiretroviral toxic neuropathy. HIV-SN patients exhibited significantly greater mean age, peak plasma viral loads, and exposure to neurotoxic dideoxynucleosides and protease inhibitors, including indinavir, saquinavir, or zidovudine. HIV-infected DRG cultures exposed to indinavir or didanosine showed significant neuronal atrophy, neurite retraction, and process loss, compared with controls. Indinavir was selectively cytotoxic to DRG macrophages compared with other ARTs.

Interpretation: Protease inhibitor exposure is an unrecognized risk factor for the development of HIV-SN, which may potentiate neuronal damage in HIV-infected DRGs, possibly through the loss of macrophage-derived trophic factors.

Ann Neurol 2006;59:816–824

Peripheral neuropathy has become the chief neurological complication observed among persons infected with human immunodeficiency virus type 1 (HIV-1) in the developed world.¹ The most common form of peripheral neuropathy in this population is a sensory polyneuropathy (HIV-SN), affecting as many as 35% of individuals with acquired immunodeficiency syndrome (AIDS). Two principal forms of HIV-SN are recognized including distal sensory polyneuropathy (DSP)² and antiretroviral-induced toxic neuropathy (ATN).³ In both types of sensory neuropathy, clinical features are defined by pain, paresthesiae, gait instability, and autonomic dysfunction. HIV-SN is characterized by prominent small-diameter axonal loss, follow-

ing a “dying back” pattern of degeneration, likely driven by local inflammation within the nerve and dorsal root ganglia (DRGs) with ensuing neuronal injury (reviewed in Pardo and colleagues⁴). Several demographic and clinical features are associated with the development of DSP including increased age, high viral load, and low CD4 counts.³ The use of specific antiretroviral drugs has been associated with the development of ATN, which is related to the use of the nucleoside analogue reverse transcriptase inhibitors (NRTI) didanosine (ddI), zalcitabine (ddC), and stavudine (d4T).⁶ ATN is indistinguishable from DSP on clinical examination except for the history of recent NRTI use.⁷ The small-diameter nociceptive sensory ax-

From the Departments of ¹Clinical Neurosciences, ²Social Work, and ³Anthropology, University of Calgary, Calgary, Alberta, Canada; ⁴Department of Virology, University of Heidelberg, Heidelberg, Germany; ⁵Department of Neurology, Johns Hopkins University, Baltimore, MD; Departments of ⁶Medicine and ⁷Microbiology and Infectious Diseases, University of Calgary, Calgary; and ⁸Department of Medicine, University of Alberta, Edmonton, Alberta, Canada.

Received Sep 14, 2005, and in revised form Jan 3, 2006. Accepted for publication Jan 7, 2006.

J.A.P. and G.J. contributed equally to this study.

Published online Apr 24, 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20816

Address correspondence to Dr Power, Department of Medicine, 6-11 Heritage Medical Research Centre, University of Alberta, Edmonton, AB T6G 2S2, Canada. E-mail: chris.power@ualberta.ca

ons and their respective soma in the DRGs are the principal cellular structures affected in HIV-induced DSP or NRTI-induced ATN.⁸ However, ATN may be a synergistic consequence of HIV infection together with the neurotoxic effects of select antiretroviral drugs, because some HIV-uninfected animals, treated with neurotoxic antiretroviral drugs, do not develop an ATN-like phenotype.⁹ Although select NRTIs are associated with ATN, multiple antiretroviral regimens including those with protease inhibitors (PIs) appear to have adverse effects on metabolism with increased risks for mitochondrial toxicity, hyperglycemia, hyperlipidemia, and the lipodystrophy syndrome.^{10,11} Given the increasing concerns regarding the prevalence of metabolic abnormalities and sensory neuropathy among an aging population of HIV/AIDS patients, we hypothesized that HIV-SN was associated with emerging clinical variables including the expanded use of different antiretroviral therapy regimens. To explore these issues, we investigated a community clinic-based cohort of HIV/AIDS patients with or without HIV-SN, showing that different ARTs including specific PIs were associated with the development of HIV-SN, and we then determined the neurotoxicity of select ARTs in a model of HIV-SN.

Patients and Methods

Clinical Investigations

STUDY SAMPLE AND DESIGN. The Southern Alberta Clinic (SAC) is a comprehensive and centralized community outpatient HIV clinic that provides all HIV seropositive patients in southern Alberta, Canada, with clinical care at regular intervals (3 months). This care includes the dispensing of antiretroviral and related medications, as well as providing laboratory investigations (CD4/CD8 lymphocyte counts, viral loads, and so forth) without patient financial cost.¹² Inclusion in this study was voluntary, and informed consent for participation was reviewed at the outset, which was approved by the University of Calgary Ethics Committee. From a cohort of more than 800 HIV-1-seropositive patients actively followed through SAC, those with neurological disorders comprised our study sample. All patients in this study were assessed by a neurologist (C.P. or A.H.) between 1998 and 2004 at the time of referral to the NeuroAIDS Clinic within SAC and were determined to have either no neuropathy (HIV-NN) or neuropathy (HIV-SN), either DSP or ATN, if all of the following three criteria were present: (1) distal sensory symptoms: paresthesias, dysesthesias, or pain; (2) abnormal sensory signs: elevated vibratory threshold or hyperalgesia; and (3) decreased or absent ankle reflexes. In addition, the diagnosis of ATN required use of antiretroviral agents with established neurotoxic effects including d4T, ddI, and ddC within the 6-month period preceding the onset of the neuropathy. Patients with no evidence of neuropathy (HIV-NN) had other neurological diagnoses (eg, HIV-associated dementia/Minor Cognitive-Motor Deficit, back pain, headache), whereas patients with

neuropathy together with other concurrent neurological disorders were included in the HIV-SN group.

Clinical data were collected prospectively from the time that the patients were initially assessed for HIV infection at SAC and then at subsequent intervals until 2004.¹³ Demographic data, HIV risk factors, and clinical data were derived from a prospectively constructed computerized database, dating back until 1991.¹⁴ Health-related quality of life was evaluated using the Medical Outcomes Short-Form Health Survey, or MOS-HIV (Version 2.97; Albert W. Wu, Johns Hopkins University, Baltimore, MD). Exposure to individual antiretroviral agents before the diagnosis of HIV-SN or other neurological conditions, including the NRTI inhibitors d4T, ddI, ddC, zidovudine (AZT), and lamivudine (3TC), and the PIs including saquinavir (SQV), ritonavir (RTV), indinavir (IDV), amprenavir (AMP), nelfinavir (NFV), and RTV-boosted lopinavir (r/LOP), was measured as exposure time (number of days), dosage (number of days multiplied by the dose), and proportion of patients within each group who had received the antiretroviral agent. Fasting plasma glucose levels (within 3 months of neuropathy onset) and the frequency of lipodystrophy were variables used in a post hoc analysis.

Laboratory Investigations

CELL CULTURES AND VIRUS INFECTION. DRGs were harvested and prepared from adult Sprague-Dawley rats, transgenic for human CD4 and CCR5, and thus permissive to HIV-1 infection.¹⁵ Culture medium was replaced after 24 hours, and then every 2 to 3 days for the next week. DRG cultures were then infected with a recombinant infectious HIV-1 clone derived from the peroneal nerve of a patient with DSP (HIV-1 p24 20ng per culture)¹⁶ or mock infected, as described previously.¹⁷ Cultures were washed after 24 hours to remove input inocula and cultured with and without ddI or IDV for a further 3 days, with medium replenished at day 2. At day 4 after infection, DRG cultures were washed, fixed with 95% ethanol, and blocked overnight at 4°C with phosphate-buffered saline containing 50% normal goat serum. After removal of the blocking reagent, the cells were immunolabeled overnight at 4°C with either mouse anti-microtubule-associated protein 2 (MAP2) (clone HM-2; 1:1,000 dilution; Sigma, St. Louis, MO), mouse anti-ED-1 (1:200 dilution; Chemicon International, Temecula, CA), mouse anti-human CD4 (1:100 dilution; DAKO, Carpinteria, CA), mouse anti-human CCR5 (cl. 2D7; 1:100 dilution; BD Biosciences, San Jose, CA), or HIV-1_{SF2} p24 rabbit antiserum (1:1,000 dilution; obtained through the AIDS Research and Reference Reagent Program, catalogue number 4250), as reported previously.¹⁸ After primary antibody staining, the cells were washed in phosphate-buffered saline and incubated with either Cy3-conjugated goat anti-mouse (1:2,000 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA) or Alex Fluor-488-conjugated goat anti-rabbit (1:2,000 dilution; Molecular Probes, Eugene, OR) secondary antibodies. In addition, DRG cultures were subjected to terminal deoxynucleotidyltransferase-mediated uridine 5'-triphosphate-biotin nick end labeling (TUNEL), as reported previously¹⁸ after treatment with AZT, IND, or ddI at a range of concentrations. Slides were mounted with Gel-

Table 1. Clinical Features of Patients with Human Immunodeficiency Virus–Associated Sensory Neuropathy and Human Immunodeficiency Virus with No Neuropathy

Variable	HIV-SN (N = 101)	HIV-NN (N = 120)	<i>p</i>
Mean age (SE)	47.2 (0.9)	43.9 (0.8)	<0.01
Sex, % male	91.1	84.2	NS
Educational level (degree), %	20.0	13.9	NS
Ethnocultural, % white	90.9	85.6	NS
MSM, %	66.3	63.9	NS
Substance use, %	30.7	39.2	NS
Alcohol use, %	11.8	23.3	<0.05
Diabetes, %	6.9	2.5	NS
Mean peak fasting glucose, mmol/L (SE) ^a	5.7 (0.09)	5.4 (0.07)	<0.01
Impaired fasting glucose: <6.1 mmol/L, % ^a	27	15	NS
Lipodystrophy, %	13	5.8	NS
Mean peak viral load, log ₁₀ copies/ml (SE)	5.1 (0.1)	4.9 (0.09)	<0.005
Mean CD4 nadir, cells/mm ³ (SE)	130.4 (15.4)	147.3 (13.2)	NS
HBV, %	29.7	27.5	NS
HCV, %	15.8	14.2	NS
TB, %	5.9	5.0	NS
Syphilis, %	7.9	8.3	NS
HAD/MCMD, %	30.7	26.7	NS
HRQoL: mean Overall Health Score (SE)	34.5 (3.2)	48.2 (3.4)	<0.05
Mean duration of HIV-1 positivity, months (SE)	90.6 (6.1)	94.3 (1.2)	NS

^aPatients with diabetes were excluded.

HIV-SN = human immunodeficiency virus–associated sensory neuropathy; HIV-NN = human immunodeficiency virus with no neuropathy; SE = standard error; NS = not significant; MSM = male-to-male sexual relations; HBV = hepatitis B virus; HCV = hepatitis C virus; TB = tuberculosis; HAD/MCMD = HIV-associated dementia/Minor Cognitive-Motor Deficit; HRQoL = health-related quality of life; HIV-1 = human immunodeficiency virus type 1.

vatol and viewed using a Zeiss Axioskop 2 upright fluorescent microscope (Zeiss, Thornwood, NY). For the DRG morphological studies, digital images of all MAP2-immunopositive neurons from 12 fields of view per well were captured using the Advanced Spot system (Diagnostic Instruments, Sterling Heights, MI), and quantitative analyses of neuronal soma size, maximal neurite lengths, and neuritic loss were performed using the Scion Image program (Scion, Frederick, MD).

STATISTICAL ANALYSES. Bivariate relationships involving discrete variables were analyzed using the χ^2 test, whereas those involving continuous variables were assessed using analysis of variance with post hoc Bonferroni, Dunn, or Tukey–Kramer comparisons or Student's *t* or Mann–Whitney *U* tests for data with or without a normal distribution, respectively. The level of significance was defined as *p* less than 0.05 for all tests.

Results

Clinical Investigations

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS. From the SAC cohort of HIV-1–positive patients, 221 were determined to have specific neurological syndromes: 120 (54%) showed no evidence of neuropathy (HIV-NN), whereas 64 (29%) and 37 (17%) exhibited either DSP or ATN, respectively, comprising those with neuropathy (HIV-SN). HIV-SN patients were significantly older than HIV-NN patients, yet this difference was not due to a longer duration of documented HIV-1

seropositivity (Table 1). Comparison of the mean duration (months) of follow-up subsequent to neurological diagnosis was 43.7 ± 20.7 (range, 2.5–73.7) months for the HIV-SN group and 37.9 ± 19.7 (range, 4.8–94.8) months for the HIV-NN group. Similar to earlier studies, mean peak viral load was higher in the HIV-SN group, whereas mean CD4 nadir level was not different between groups (see Table 1). Not unexpectedly, quality of life as measured by the Overall Health Score was significantly lower among the HIV-SN patients compared with the HIV-NN group. Other demographic variables, including sex, educational level, male-to-male sexual relations, and substance use did not differ between the HIV-SN and HIV-NN groups, nor did the presence of various coinfections (hepatitis B virus, hepatitis C virus, tuberculosis, syphilis) or cognitive impairment (HIV-associated dementia or Minor Cognitive-Motor Deficit). In addition, mean CD4 and CD8 levels in blood closest to the time of HIV seropositivity or neurological diagnosis and mean log viral load at the time neurological diagnosis did not differ between the HIV-NN and HIV-SN groups, although gabapentin use as an analgesic was significantly higher in the HIV-SN group (*p* < 0.01). However, after stratification by ATN versus DSP (Table 2), patients with ATN were more likely to have had male-to-male sexual relations and higher levels of education, whereas the risk for HCV

Table 2. Clinical Features of Patients with Distal Sensory Polyneuropathy and Antiretroviral-Induced Toxic Neuropathy

Variable	DSP (N = 64)	ATN (N = 37)	<i>p</i>
Mean age (SE)	46.2 (0.92)	49.0 (1.9)	NS
Sex, % male	91.5	91.9	NS
Educational level (degree), %	12.3	33.3	<0.05
MSM, %	57.8	81.1	<0.05
Substance use, %	35.9	21.6	NS
Alcohol use, %	33.9	50.0	NS
Diabetes, %	10.9	5.4	NS
Mean peak viral load, log ₁₀ copies/ml (SE)	5.16 (0.11)	5.11 (0.19)	NS
Mean CD4 nadir, cells/mm ³ (SE)	139.3 (20.2)	140.0 (23.5)	NS
HBV, %	28.1	32.4	NS
HCV, %	21.9	5.4	<0.05
TB, %	7.8	2.7	NS
Syphilis, %	3.1	16.2	<0.05
HAD/MCMD, %	34.4	24.3	NS
Mean duration of HIV-1 seropositivity, months (SE)	88.8 (8.1)	93.5 (9)	NS
Neurotoxic ART exposure, %	71.9	100.0	<0.001

DSP = distal sensory polyneuropathy; ATN = antiretroviral-induced toxic neuropathy; SE = standard error; NS = not significant; MSM = male-to-male sexual relations; HBV = hepatitis B virus; HCV = hepatitis C virus; TB = tuberculosis; HAD/MCMD = HIV-associated dementia/Minor Cognitive-Motor Deficit; HRQoL = health-related quality of life; HIV-1 = human immunodeficiency virus type 1; ART = antiretroviral therapy.

infection was less compared with the DSP group ($p < 0.05$), although the frequency of syphilis was higher in the ATN group ($p < 0.05$).

ANTIRETROVIRAL THERAPY EXPOSURE. The use of individual neurotoxic dideoxynucleosides including d4T, ddC, and ddI was significantly associated with the presence of HIV-SN (Fig 1), as shown by the proportion of patients exposed to each agent (see Fig 1A), the number of days exposed to each agent (see Fig 1B), and the total dosage of each drug (data not shown; $p < 0.05$). Indeed, the risk for development of ATN was higher among patients who received any of the previously recognized neurotoxic ARTs (see Table 2; $p < 0.001$). The use of PIs was also significantly associated with the presence of HIV-SN by the proportion of patients (see Fig 1A) and the total number of days exposed to PIs (see Fig 1B). Similarly, cumulative PI dosage was significantly greater in those who had developed HIV-SN (data not shown; $p < 0.005$). Concentrating on PI use, it was observed that the development of HIV-SN was significantly associated with the cumulative exposure of certain PIs including IDV, SQV, and RTV (see Fig 1C). Moreover, the number of patients receiving IDV (60.3% HIV-SN vs 35.0% HIV-NN; $p < 0.0005$), SQV (49.5% HIV-SN vs 25.8% HIV-NN; $p < 0.0005$), or RTV (48.5.3% HIV-SN vs 27.5% HIV-NN; $p < 0.005$) differed between groups, although HIV-SN frequency was not associated with AMP, r/LOP, and NFV use. In addition, the number of days that the HIV-SN group received IDV, SQV, or RTV was also higher than the HIV-NN group (data not shown; $p < 0.005$), whereas again,

AMP, r/LOP, and NFV exposure times were not related to the development of HIV-SN. Importantly, both HIV-SN groups (ATN and DSP) did not differ in their comparative PI exposure times, cumulative dosages, or proportions of patients exposed to these agents (data not shown).

As the association between PI use and the development of HIV-SN might be related to glucose intolerance or undetected diabetes, we assessed fasting plasma glucose levels from within 3 months of the diagnosis of HIV-SN after excluding those patients with diabetes mellitus. Mean peak fasting glucose (\pm standard error) was significantly higher in the HIV-SN group compared with the HIV-NN group (5.7 ± 0.09 vs 5.4 ± 0.07 mmol/L, respectively; $p < 0.01$). However, the frequency of patients with impaired glucose tolerance (defined as >6.1 mmol/L) did not differ between groups (see Table 1). Although there was a trend toward a higher prevalence of lipodystrophy in the HIV-SN group, this difference was not statistically significant (see Table 1).

Laboratory Investigations

DRG cultures were composed of neurons, Schwann cells (not shown), and human CD4- and CCR5-positive macrophages (Fig 2A). After HIV-1 infection, HIV-1 p24 was colocalized with the rat macrophage marker ED-1 (see Fig 2B), which was accompanied by neurite retraction and neuronal soma atrophy. Quantitation of mean neurite length (see Fig 2C), mean number of neurons with processes (see Fig 2D), and mean neuronal soma size (see Fig 2E) showed that HIV infection and IDV treatment

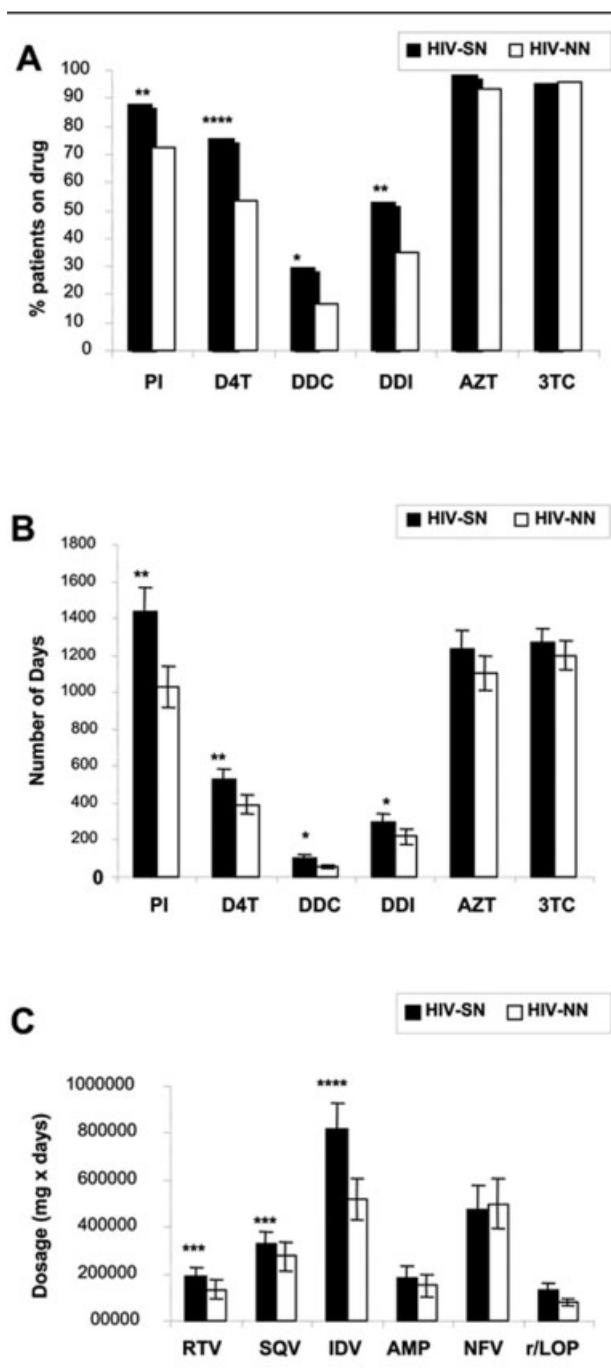


Fig 1. Antiretroviral therapy (ART) exposure in patients with human immunodeficiency virus–associated sensory neuropathy (HIV-SN; black bars) and HIV with no neuropathy (HIV-NN; open bars). (A) Percentage of patients exposed to each ART agent. (B) Mean total number of days (\pm standard deviation [SD]) of exposure to each agent. (C) Mean total dosage (\pm SD; dose of each agent multiplied by the total number of days) for each of the protease inhibitors (PIs). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$. AZT = zidovudine; d4T = stavudine; ddC = zalcitabine; ddI = didanosine; 3TC = lamivudine.

(0.1 μ M) of mock-infected cultures caused nonsignificant reductions in all of these neuronal parameters. Treatment of mock-infected cultures with ddI (1.0 μ M) resulted in a significant reduction in both neurite length ($p < 0.01$) and neuronal soma size ($p < 0.001$), similar to our previous findings with other NRTIs.¹⁹ However, in cultures infected with HIV-1 with subsequent treatment by ddI or IDV, there was a marked reduction in all neuronal parameters compared with mock- and HIV-infected cultures, suggesting that select ARTs exert additive neurotoxic effects in this model of HIV-SN. To determine whether certain cell types were selectively injured by IDV or ddI treatment at clinically relevant concentrations, we examined DRG cultures after TUNEL and found that untreated cultures (Fig 3A) showed no TUNEL positivity. Conversely, IDV-treated cultures (1.0 and 100.0 μ M) showed numerous TUNEL-positive cells (see Fig 3B). Double immunolabeling showed colocalization of TUNEL and ED-1 immunoreactivity (see Fig 3B, inset) although TUNEL was not colocalized with MAP2 immunoreactivity (data not shown). Quantitation of the number of TUNEL-positive cells disclosed that there was a significant increase in TUNEL positivity in IDV-treated cultures compared with mock-, ddI- and AZT-treated cultures. These results implied that monocytoic cells may be targets of IDV toxicity in keeping with previous studies,²⁰ and that macrophages are necessary for neuronal viability.

Discussion

In this cohort study of HIV-1–seropositive patients, we identified several distinguishing demographic and clinical features of HIV-SN. In particular, certain PIs, including IDV, SQV, and RTV, exhibited greater cumulative dosages and exposure times in patients who developed HIV-SN. Interestingly, these same three agents penetrate neural compartments most efficiently of all the PIs,^{21–23} leading us to hypothesize that they may have more direct neurotoxic effects in a model of HIV-SN. The latter investigations indicated that both IDV and, as expected, ddI were highly neurotoxic during concurrent HIV infection. Age, alcohol use, peak viral load, and quality of life also distinguished patients with and without HIV-SN. That older age was associated with neuropathy is not unique to our study and has been reported previously.^{24–27} However, this finding could not be attributed to longer duration of HIV-1 seropositivity, as has been suggested earlier.²⁵ Indeed, age is a risk factor for other neurological manifestations of HIV-1, including HIV-associated dementia and its antecedent condition (Minor Cognitive-Motor Deficit), together with progressive multifocal leukoencephalopathy, primary central nervous system lymphoma, and stroke.²⁴ Previous studies have re-

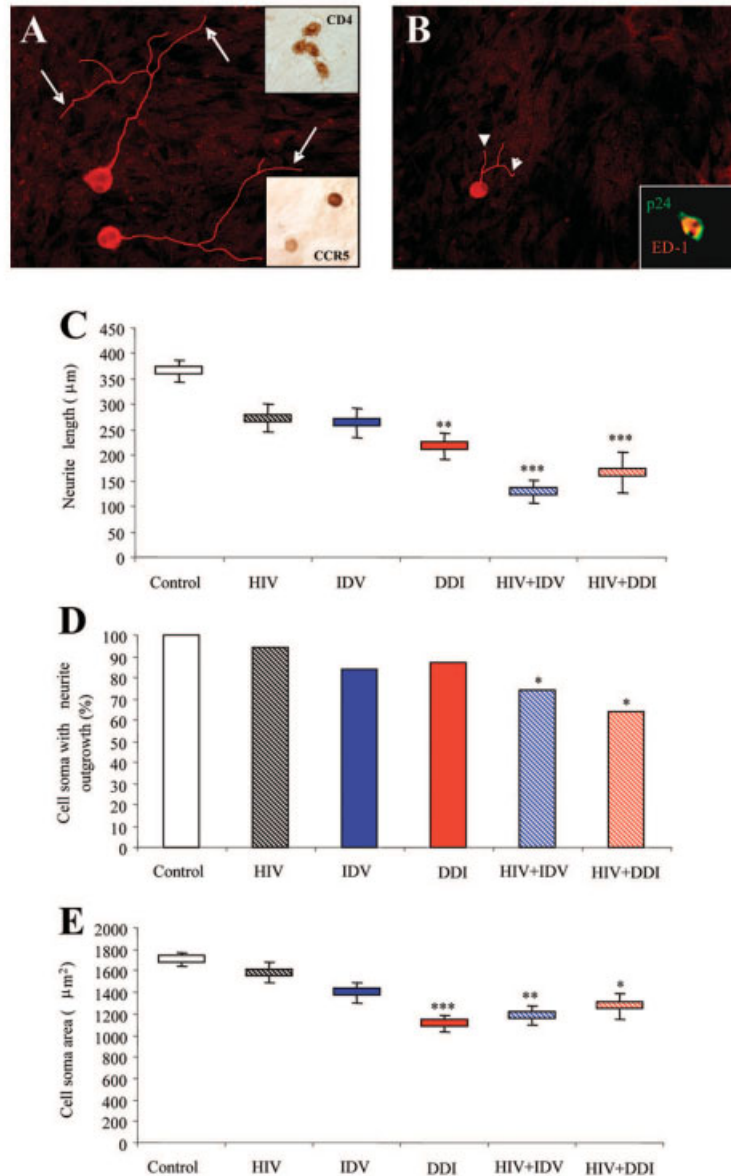


Fig 2. Dorsal root ganglion (DRG) cultures from human CD4/CCR5 transgenic rats. (A) Microtubule-associated protein 2 (MAP2)-immunopositive neurons (red) with extensive processes (arrows) together with CD4- (inset, brown) and CCR5 (inset, brown)-immunopositive macrophages. (B) Human immunodeficiency virus (HIV)-infected DRG cultures exhibited shortened and less processes (arrowheads) with cell atrophy, and HIV p24 (inset, yellow) was detectable in ED-1-immunopositive macrophages. Whereas HIV infection and indinavir treatment (IDV) showed nonsignificant neuronal changes (C–E), the combination of HIV infection with IDV (0.1µM) or didanosine (ddI; 1.0µM) treatments resulted significant reductions in (C) mean neurite length (\pm standard deviation [SD]), (D) mean number of neurons with processes (\pm SD), with (E) mean neuronal soma diameter (\pm SD). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

ported either a positive association between alcohol consumption and the development of HIV-SN^{26,28} or no alcohol effects²⁹; thus, it appears counterintuitive that alcohol use was more prevalent among those without HIV-SN in this study. Conversely, alcohol has been shown to inhibit neuronal injury in models of HIV gp120 neurotoxicity.³⁰ Although the underlying mechanisms for this neuroprotective effect by alcohol

have not been fully elucidated, impaired secretion of proinflammatory cytokines (eg, tumor necrosis factor- α , interleukins) and promotion of endogenous protective factors such as transforming growth factor- β have been suggested.³⁰ Regarding HIV-SN, a similar neuroprotective effect of alcohol on the peripheral nervous system remains possible, though speculative. A higher peak viral load was associated with HIV-SN,

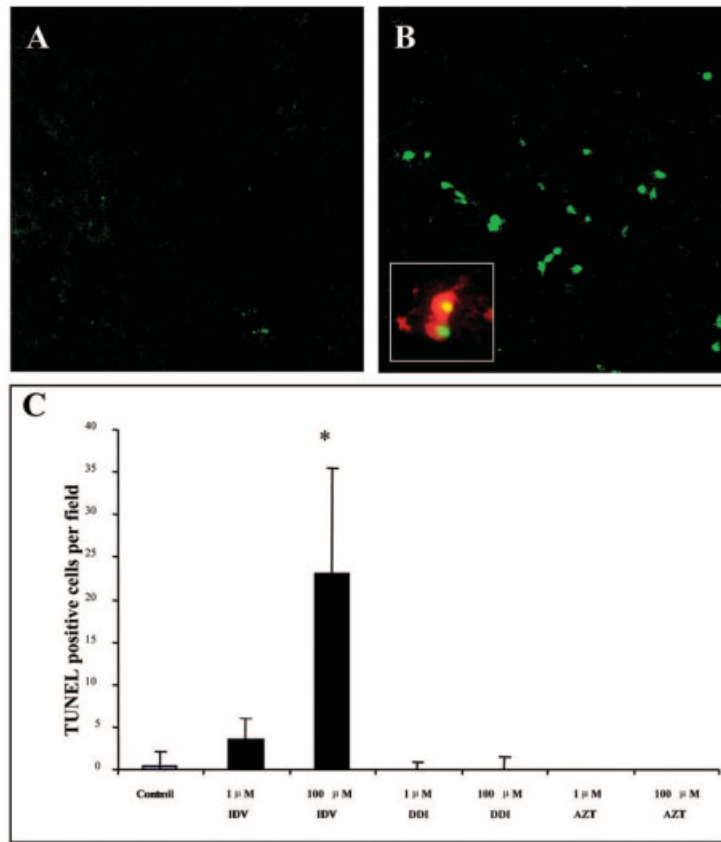


Fig 3. Terminal deoxynucleotidyltransferase-mediated uridine 5'-triphosphate-biotin nick end labeling (TUNEL) in dorsal root ganglion (DRG) cultures treated with antiretroviral therapies (ARTs). (A) Mock-treated cultures exhibited no TUNEL-positive cells. (B) Indinavir (IDV)-treated cultures showed numerous TUNEL-positive nuclei in cells that were ED-1-immunopositive (inset). (C) Quantitation of TUNEL detection showed that IDV-treated cultures contained significantly high numbers of TUNEL-positive cells compared with mock-, zidovudine (AZT)-, and didanosine (ddI)-treated cultures. $**p < 0.001$.

whereas a lower CD4 count was not. The previously reported literature assessing immune status and HIV-SN is mixed, with some prior reports linking neuropathy to increased viral load levels,^{5,31} whereas others have reported no relationship.³² Although lower CD4 counts have also been associated with neuropathy,^{5,25,27,33,34} there has been a trend over time toward the development of HIV-SN at higher CD4 levels than previously observed.^{25,28} Morgello and colleagues²⁸ speculate that this difference in findings relates to a difference in patient populations (ie, earlier reports predated the availability of highly active antiretroviral therapy). Perhaps not unexpectedly, patients with HIV-SN were shown to have a lower overall health score on the quality of life index. This latter finding is likely related to increased pain and sensory impairments associated with this type of neuropathy, as evidenced by greater gabapentin use reported herein. Notably, the present definition of HIV-SN required patients to be symptomatic from their neuropathy for inclusion, which may have biased our study toward HIV-SN patients with more severe neuropathy.

Nonetheless, d4T, ddC, and ddI exposure within 6 months of diagnosis were also shown to be significantly associated with the development of HIV-SN, confirming earlier findings that these three agents are neurotoxic.^{3,7,35,36} A putative mechanism underlying this toxicity is altered neuronal mitochondrial metabolism.^{7,37}

In this cohort of HIV-infected patients, we identified an association between specific PIs and the development of HIV-SN. Concordant results on all three measures of PI exposure were observed including proportion of patients exposed to PIs, total number of days receiving PIs, and the total dosage of PIs taken. A recent report indicates that LOP, IDV, RTV, and SQV, but not NFV, were associated with neuropathy in HIV/AIDS patients.³⁸ However, the same study reported AZT, efavirenz, and lamivudine were also associated with neuropathy, although these drugs have not previously been implicated in neuropathy. Indeed, this represents a limitation to our study in that our patient groups were smaller than those in the latter study,³⁸ albeit our patients were more intensively examined, but

there may also have been some inherent biases as the HIV-NN group had other neurological disorders. The association between HIV-SN and PI use has otherwise not been recognized in other studies, perhaps due to insufficient duration of follow-up or merely limited databases. PIs became available to all patients in our clinic in late 1996, which was recorded prospectively in our computerized database, although this might have skewed our data toward PI-related HIV-SN for those PIs used more intensively over time. The occurrence of abnormal fasting glucose tests did not differ in HIV-SN and HIV-NN groups, despite modest differences in peak fasting glucose levels; hence, these findings suggest that incipient diabetes was not a factor distinguishing the HIV-SN and HIV-NN groups. In our analysis, IDV, SQV, and RTV were significantly associated with HIV-SN, and interestingly, all of these PIs have been previously shown to penetrate the blood-brain barrier^{32,39,40} and in the case of IDV is actively transported into the CSF.⁴¹ Likewise, these PIs may also penetrate the DRGs, raising the possibility of direct toxicity to DRG neurons because the DRG represents a susceptible target for any circulating neurotoxin (or drug) because its blood-nerve barrier is a highly permeable region within the peripheral nerve.⁴²

These laboratory studies, using a novel model of neuronal injury in HIV-SN, support our clinical findings by indicating that although infection per se by a peripheral nerve-derived HIV clone may affect neuronal viability to a limited extent, the concomitant use of IDV clearly exacerbated neuronal injury. Not unexpectedly, ddI was also neurotoxic to DRG neurons, in keeping with our earlier results.¹⁹ Neurite retraction and less neurites were evident in both IDV- and ddI-treated cultures infected by HIV-1. The concentrations of IDV used herein for DRG toxicity studies ranged from 0.1 to 100.0 μM , whereas trough IDV levels detected in serum are 1.0 to 10 μM and in CSF 0.1 to 1.0 μM , depending on the individual study.^{43,44} Hence, peak serum and cumulative tissue concentrations of IDV may approximate the drug concentrations used in these studies. These findings, together with that of reduced neuronal soma size, also imply that neuronal dysfunction, perhaps preapoptotic,⁴⁵ may be the basis of HIV-SN induced by PIs that is a potentially reversible process. Indeed, cessation of neurotoxic NRTIs and improved immune status frequently lead to a resolution in symptoms, if not signs, of HIV-SN.⁴⁶ Interestingly, we found that IDV was selectively cytotoxic to macrophages in this culture system. This observation suggests that macrophages contribute to neuronal maintenance possibly through the release of neurotrophic factors including nerve growth factor, insulin-like growth factor-1, and brain-derived neurotrophic factor.^{47,48} HIV infection in conjunction with IDV-related toxicity likely results in a marked reduc-

tion in macrophage viability with ensuing adverse effects on neurons. Given the widespread and protracted use of PIs, these findings indicate there is an urgent need to define thresholds of neurotoxicity for PI dosage and exposure times, thereby preventing the development of HIV-SN among the growing number of HIV/AIDS patients receiving antiretroviral therapy.

This study was supported by the Canadian Institutes for Health Research (CIHR; C.P.), the NIH (National Institute of Neurological Disorders and Stroke, IR 01NS4626201, C.P.; R00051-B, O.T.K.), a CIHR Fellowship (G.J.), and a Canada Research Chair (Tier 1) in Neurological Infection and Immunity (C.P.).

We thank D. Zochodne for helpful discussions, B. Ibrahim and S. Sweeney for manuscript preparation, and the J. David Gladstone Institutes for providing the human CD4/CCR5-transgenic rats.

References

1. Brew BJ. The peripheral nerve complications of human immunodeficiency virus (HIV) infection. *Muscle Nerve* 2003;28:542–552.
2. Leger JM, Bouche P, Bolgert F, et al. The spectrum of polyneuropathies in patients infected with HIV. *J Neurol Neurosurg Psychiatry* 1989;52:1369–1374.
3. Simpson DM, Tagliati M. Nucleoside analogue-associated peripheral neuropathy in human immunodeficiency virus infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;9:153–161.
4. Pardo CA, McArthur JC, Griffin JW. HIV neuropathy: insights in the pathology of HIV peripheral nerve disease. *J Peripher Nerv Syst* 2001;6:21–27.
5. Childs EA, Lyles RH, Selnes OA, et al. Plasma viral load and CD4 lymphocytes predict HIV-associated dementia and sensory neuropathy. *Neurology* 1999;52:607–613.
6. Schifitto G, McDermott MP, McArthur JC, et al. Markers of immune activation and viral load in HIV-associated sensory neuropathy. *Neurology* 2005;64:842–848.
7. Dalakas MC. Peripheral neuropathy and antiretroviral drugs. *J Peripher Nerv Syst* 2001;6:14–20.
8. McCarthy BG, Hsieh ST, Stocks A, et al. Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. *Neurology* 1995;45:1848–1855.
9. Anderson TD, Davidovich A, Arceo R, et al. Peripheral neuropathy induced by 2',3'-dideoxycytidine. A rabbit model of 2',3'-dideoxycytidine neurotoxicity. *Lab Invest* 1992;66:63–74.
10. Tsiodras S, Mantzoros C, Hammer S, Samore M. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. *Arch Intern Med* 2000;160:2050–2056.
11. Carr A, Samaras K, Thorisdottir A, et al. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet* 1999;353:2093–2099.
12. Kim D, Jewison DL, Milner GR, et al. HIV-related neurocognitive impairment in patients receiving highly active antiretroviral therapy, HAART. *Can J Neurol Sci* 2001;28:228–231.
13. van Marle G, Rourke SB, Zhang K, et al. HIV dementia patients exhibit reduced viral neutralization and increased envelope sequence diversity in blood and brain. *AIDS* 2002;16:1905–1914.
14. Krentz HB, Auld MC, Gill MJ. The changing direct costs of medical care for patients with HIV/AIDS, 1995–2001. *Can Med Assoc J* 2003;169:106–110.

15. Keppler OT, Welte FJ, Ngo TA, et al. Progress toward a human CD4/CCR5 transgenic rat model for de novo infection by human immunodeficiency virus type 1. *J Exp Med* 2002;195:719–736.
16. Jones G, Zhu Y, Silva C, et al. Peripheral nerve-derived HIV-1 is predominantly CCR5-dependent and causes neuronal degeneration and neuroinflammation. *Virology* 2005;334:178–193.
17. Power C, McArthur JC, Nath A, et al. Neuronal death induced by brain-derived human immunodeficiency virus type 1 envelope genes differs between demented and nondemented AIDS patients. *J Virol* 1998;72:9045–9053.
18. Zhang K, McQuibban GA, Silva C, et al. HIV-induced metalloproteinase processing of the chemokine stromal cell derived factor-1 causes neurodegeneration. *Nat Neurosci* 2003;6:1064–1071.
19. Keswani SC, Chander B, Hasan C, et al. FK506 is neuroprotective in a model of antiretroviral toxic neuropathy. *Ann Neurol* 2003;53:57–64.
20. Estaquier J, Lelievre JD, Petit F, et al. Effects of antiretroviral drugs on human immunodeficiency virus type 1-induced CD4(+) T-cell death. *J Virol* 2002;76:5966–5973.
21. Anthonypillai C, Sanderson RN, Gibbs JE, Thomas SA. The distribution of the HIV protease inhibitor, ritonavir, to the brain, cerebrospinal fluid, and choroid plexuses of the guinea pig. *J Pharmacol Exp Ther* 2004;308:912–920.
22. Isaac A, Taylor S, Cane P, et al. Lopinavir/ritonavir combined with twice-daily 400 mg indinavir: pharmacokinetics and pharmacodynamics in blood, CSF and semen. *J Antimicrob Chemother* 2004;54:498–502.
23. Kravcik S, Gallicano K, Roth V, et al. Cerebrospinal fluid HIV RNA and drug levels with combination ritonavir and saquinavir. *J Acquir Immune Defic Syndr* 1999;21:371–375.
24. Goodkin K, Wilkie FL, Concha M, et al. Aging and neuro-AIDS conditions and the changing spectrum of HIV-1-associated morbidity and mortality. *J Clin Epidemiol* 2001;54(suppl 1):S35–S43.
25. Maschke M, Kastrup O, Esser S, et al. Incidence and prevalence of neurological disorders associated with HIV since the introduction of highly active antiretroviral therapy (HAART). *J Neurol Neurosurg Psychiatry* 2000;69:376–380.
26. Lopez OL, Becker JT, Dew MA, Caldararo R. Risk modifiers for peripheral sensory neuropathy in HIV infection/AIDS. *Eur J Neurol* 2004;11:97–102.
27. Tagliati M, Grinnell J, Godbold J, Simpson DM. Peripheral nerve function in HIV infection: clinical, electrophysiologic, and laboratory findings. *Arch Neurol* 1999;56:84–89.
28. Morgello S, Estanislao L, Simpson D, et al. HIV-associated distal sensory polyneuropathy in the era of highly active antiretroviral therapy: the Manhattan HIV Brain Bank. *Arch Neurol* 2004;61:546–551.
29. Marra CM, Boutin P, Collier AC. Screening for distal sensory peripheral neuropathy in HIV-infected persons in research and clinical settings. *Neurology* 1998;51:1678–1681.
30. Collins MA, Neafsey EJ, Zou JY. HIV-1 gp120 neurotoxicity in brain cultures is prevented by moderate ethanol pretreatment. *Neuroreport* 2000;11:1219–1222.
31. Simpson DM, Haidich AB, Schifitto G, et al. Severity of HIV-associated neuropathy is associated with plasma HIV-1 RNA levels. *AIDS* 2002;16:407–412.
32. Brew BJ, Tisch S, Law M. Lactate concentrations distinguish between nucleoside neuropathy and HIV neuropathy. *AIDS* 2003;17:1094–1096.
33. Barohn RJ, Gronseth GS, LeForce BR, et al. Peripheral nervous system involvement in a large cohort of human immunodeficiency virus-infected individuals. *Arch Neurol* 1993;50:167–171.
34. Schifitto G, McDermott MP, McArthur JC, et al. Incidence of and risk factors for HIV-associated distal sensory polyneuropathy. *Neurology* 2002;58:1764–1768.
35. Keswani SC, Pardo CA, Cherry CL, et al. HIV-associated sensory neuropathies. *AIDS* 2002;16:2105–2117.
36. Moyle GJ, Sadler M. Peripheral neuropathy with nucleoside antiretrovirals: risk factors, incidence and management. *Drug Saf* 1998;19:481–494.
37. Cossarizza A, Moyle G. Antiretroviral nucleoside and nucleotide analogues and mitochondria. *AIDS* 2004;18:137–151.
38. Lichtenstein KA, Armon C, Baron A, et al. Modification of the incidence of drug-associated symmetrical peripheral neuropathy by host and disease factors in the HIV outpatient study cohort. *Clin Infect Dis* 2005;40:148–157.
39. Letendre SL, Capparelli EV, Ellis RJ, McCutchan JA. Indinavir population pharmacokinetics in plasma and cerebrospinal fluid. The HIV Neurobehavioral Research Center Group. *Antimicrob Agents Chemother* 2000;44:2173–2175.
40. Wynn HE, Brundage RC, Fletcher CV. Clinical implications of CNS penetration of antiretroviral drugs. *CNS Drugs* 2002;16:595–609.
41. Martin C, Sonnerborg A, Svensson JO, Stahle L. Indinavir-based treatment of HIV-1 infected patients: efficacy in the central nervous system. *AIDS* 1999;13:1227–1232.
42. Seitz RJ, Heininger K, Schwendemann G, et al. The mouse blood-brain barrier and blood-nerve barrier for IgG: a tracer study by use of the avidin-biotin system. *Acta Neuropathol (Berl)* 1985;68:15–21.
43. Antinori A, Perno CF, Giancola ML, et al. Efficacy of cerebrospinal fluid (CSF)-penetrating antiretroviral drugs against HIV in the neurological compartment: different patterns of phenotypic resistance in CSF and plasma. *Clin Infect Dis* 2005;41:1787–1793.
44. Solas C, Lafeuillade A, Halfon P, et al. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 2003;47:238–243.
45. Bradley WG, Shapshak P, Delgado S, et al. Morphometric analysis of the peripheral neuropathy of AIDS. *Muscle Nerve* 1998;21:1188–1195.
46. Markus R, Brew BJ. HIV-1 peripheral neuropathy and combination antiretroviral therapy. *Lancet* 1998;352:1906–1907.
47. Barouch R, Appel E, Kazimirsky G, Brodie C. Macrophages express neurotrophins and neurotrophin receptors. Regulation of nitric oxide production by NT-3. *J Neuroimmunol* 2001;112:72–77.
48. Garaci E, Caroleo MC, Aloe L, et al. Nerve growth factor is an autocrine factor essential for the survival of macrophages infected with HIV. *Proc Natl Acad Sci U S A* 1999;96:14013–14018.