The Protective Effects of IGF-1 on Different Subpopulations of DRG Neurons with Neurotoxicity Induced by gp120 and Dideoxycytidine In Vitro

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Abstract
Peripheral neuropathy induced by human immunodeficiency virus (HIV) infection and antiretroviral therapy is not only difficult to distinguish in clinical practice, but also difficult to relieve the pain symptoms by analgesics because of the severity of the disease at the later stage. Hence, to explore the mechanisms of HIV-related neuropathy and find new therapeutic options are particularly important for relieving neuropathic pain symptoms of the patients. In the present study, primary cultured embryonic rat dorsal root ganglion (DRG) neurons were used to determine the neurotoxic effects of HIV-gp120 protein and/or antiretroviral drug dideoxycytidine (ddC) and the therapeutic actions of insulin-like growth factor-1 (IGF-1) on gp120- and ddC-induced neurotoxicity. DRG neurons were exposed to gp120 (500 pmol/L), ddC (50 μmol/L), gp120 (500 pmol/L) plus ddC (50 μmol/L), gp120 (500 pmol/L) plus IGF-1 (20 nmol/L), ddC (50 μmol/L) plus IGF-1 (20 nmol/L), gp120 (500 pmol/L) plus ddC (50 μmol/L) plus IGF-1 (20 nmol/L), respectively, for 72 hours. The results showed that gp120 and/or ddC caused neurotoxicity of primary cultured DRG neurons. Interestingly, the severity of neurotoxicity induced by gp120 and ddC was different in different subpopulation of DRG neurons. gp120 mainly affected large diameter DRG neurons (>25 μm), whereas ddC mainly affected small diameter DRG neurons (≤25 μm). IGF-1 could reverse the neurotoxicity induced by gp120 and/or ddC on small, but not large, DRG neurons. These data provide new insights in elucidating the pathogenesis of HIV infection- or antiretroviral therapy-related peripheral neuropathy and facilitating the development of novel treatment strategies.

Key Words: gp120, Dideoxycytidine, Neurotoxicity, Insulin-like growth factor-1, Neuron, Dorsal root ganglion

INTRODUCTION
Peripheral polyneuropathies are common neurologic complications related to human immunodeficiency virus (HIV) and antiretroviral therapy (Luma et al., 2012; Kokotis et al., 2013). Distal symmetric polyneuropathy (DSP) related to HIV is one of the most common neurologic complications of HIV, possibly affecting as many as 50% of all individuals infected with HIV (Huang et al., 2013; Schütz and Robinson-Papp, 2013). DSP is one of the few that are specific to HIV infection or its treatment (Gabbai et al., 2013). Mechanisms responsible for this disease are incompletely understood (Ng et al., 2011). gp120 is an envelope glycoprotein of HIV, which appears to contribute to the painful neuropathy related to HIV (Smith, 2011). Not only peripheral gp120 application into the rat sciatic nerve in vivo could cause neuropathic pain (Zheng et al., 2011), but gp120 could induce neurotoxicity of dorsal root ganglion (DRG) neurons in vitro as well (Robinson et al., 2007). It is clear that several members of the dideoxynucleoside family of nucleoside-analogue reverse transcriptase inhibitors (NRTIs) used in antiretroviral therapy are significantly neurotoxic (Höschele, 2006). 2',3'-dideoxycytidine (zalcitabine, ddC) has...
the greatest risk of developing neuropathy among NRTIs (Dallakas et al., 2001; Robinson et al., 2007). Our latest research has shown that ddC could induce neurotoxicity of specific tyrosine kinase receptor (Trk) subpopulations of DRG neurons (Liu et al., 2014).

DRG neuronal damage during lentivirus infections leading to the development of DSP (Acharjee et al., 2011). It has been shown in the latest research that HIV infection itself or antiretroviral therapy may cause different patterns of polyneuropathy. Antiretroviral therapy appears to primarily impair thin fiber conduction, whereas HIV infection itself is linked to large fiber impairment (Kokotis et al., 2013). Currently, two potentially neurotoxic mechanisms have been proposed to play a crucial role in the pathogenesis of HIV DSP: neurotoxicity resulting from the virus and its products, as well as adverse neurotoxic effects of medications used in the treatment of HIV, however, the precise mechanisms of DSP are still unclear. The progressively developed pain symptoms may interfere significantly with the patient's daily activities and quality of life. Topical or systemic administration of analgesics may help relieve neuropathic pain, however, it is difficult to relieve the pain symptoms by analgesics because of the severity of the disease at the later stage. Establishing etiology of the neurological complications and proposing novel therapeutic strategies can present a significant clinical challenge. Hence, to explore the mechanisms of HIV-related neuropathy and find new therapeutic options are particularly important for relieving neuropathic pain symptoms of these patients.

Insulin-like growth factor-1 (IGF-1) is a polypeptide growth factor with a variety of functions in neurons (Wood et al., 2007). IGF-1 has potent neurotrophic and neuroprotective properties (Lunn et al., 2010). Whether IGF-1 produces neuroprotective effects on distinct subpopulations of DRG neurons with gp120 and/or ddC-induced neurotoxicity is still to be clarified. In the present study, it is hypothesized that HIV-gp120 protein and nucleoside-analogue reverse transcriptase inhibitors (NRTI) antiretroviral drug may have neurotoxicity on different subpopulations of DRG neurons and the neuroprotective agent IGF-1 may relieve gp120- or ddC-induced neurotoxicity of different subpopulations of sensory neurons. Primary cultured embryonic rat DRG neurons were used to determine the neurotoxic effects of gp120 and/or ddC and the therapeutic actions of IGF-1 on gp120- or ddC-induced neurotoxicity. The data of the present study may facilitate the development of novel strategies for treatment of the HIV-related neuropathy, a medical condition for which few effective therapeutic options are available.

**MATERIALS AND METHODS**

**DRG cell culture preparations**

The animals were obtained from the Experimental Animal Center of Shandong University of China. All procedures described herein were reviewed by and had prior approval by the Ethical Committee for Animal Experimentation of the Shandong University. All surgery was performed under anesthesia, and all efforts were made to minimize suffering. DRG was dissected out from Wistar rat on embryonic day (E) 15. DRG prior to establishment in culture was digested with 0.25% trypsin (Sigma, St. Louis, MO, USA) in D-Hanks solution at 37°C for 10 minutes. Following digestion and dissociation, fetal bovine serum was added to 10% to stop digestion and the cells were centrifuged for 5 minutes at 1×10^3 rpm. The supernatants were removed and the pellets were resuspended in Dulbecco’s Modified Eagle Medium with F-12 supplement (DMEM/F-12) media (Gibco, Grand Island, NY, USA) and triturated using a sterile modified Pasteur's glass pipette. Cells were then filtered using a 130 μm filter followed by counting. Dissociated DRG cells were plated at 2.5×10^6 cells/well in a volume of 1.0 ml for 24-well clusters (Costar, Corning, NY, USA) precoated with poly-L-lysine (0.1 mg/ml, Sigma, St. Louis, MO, USA). Then DRG cells were cultured in culture media at 37°C with 5% CO₂ for 24 hours and then maintained in culture media containing cytosine arabinoside (5 μg/ml) for another 24 hours to inhibit growth of non-neuronal cells, and then cultured in culture media for an additional 72 hours in different experimental conditions before observation. The composition of the culture media is D-MEM/F-12 (1:1) supplemented with 5% fetal bovine serum, 2% B-27 supplement (Gibco, Grand Island, NY, USA) without antioxidants, L-glutamine (0.1 mg/ml, Sigma, St. Louis, MO, USA).

**Exposure of different agents on DRG cultures**

At 48 hours post-culture, primary cultured DRG neurons were exposed to gp120 (500 pmol/L, Sigma, St. Louis, MO, USA), ddC (50 μmol/L, Sigma, St. Louis, MO, USA), gp120 (500 pmol/L)+ddC (50 μmol/L), gp120 (500 pmol/L)+IGF-1 (20 nmol/L, PeproTech Inc., Pocky Hill, NJ, USA), ddC (50 μmol/L)+IGF-1 (20 nmol/L), gp120 (500 pmol/L)+ddC (50 μmol/L)+IGF-1 (20 nmol/L), respectively, for an additional 72 hours. DRG neurons were cultured continuously in culture medium served as a control.

**Total neurite length measurement of different subpopulation of neurons**

After treatment with different agents for 72 hours, DRG neuronal cultures were processed for fluorescent labeling of microtubule-associated protein 2 (MAP2). The cells on coverslips were quickly rinsed once in 0.1 mol/L phosphate buffer saline (PBS) to remove media. The cells were fixed in 4% paraformaldehyde, pH 7.4, for 20 minutes at 4°C. After washing in 0.1 mol/L PBS for 3 times, the cells were blocked with 2% normal goat serum in 0.6% Triton PBS to block non-specific sites and permeabilize the cells. The samples were incubated with mouse monoclonal anti-MAP2 (1:400, Abcam, Cambridge, MA, USA) overnight at 4°C. After washing in 0.1 mol/L PBS 3 times, the cells were incubated with goat anti-mouse conjugated to Cy2 (1:200, Abcam, Cambridge, MA, USA) for 45 minutes in darkness. After washing in 0.1 mol/L PBS, the coverslips were placed on glass slides immediately with anti-fade mounting medium (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and stored at 4°C prior to observation with a fluorescent microscope. All labeled DRG neurons were defined as small neurons (the diameter of neuronal cell body ≤25 μm) and large neurons (the diameter of neuronal cell body >25 μm). The total neurite length of each small neuron and large neuron was measured separately. Total neurite length was measured using ImageJ software in a double blind manner by random focusing.

**Double fluorescent labeling of MAP2 and GAP-43**

At 72 hours post-exposure of different agents, dissociate DRG cultures were processed for double immunofluorescent labeling with anti-MAP2 and anti-GAP-43.
labeling of MAP2 and growth-associated protein 43 (GAP-43). The cells on coverslips were rinsed quickly once in 0.1 mol/L PBS to remove media. Then, cells were fixed in 4% paraformaldehyde, pH 7.4, for 40 minutes at 4°C. After washing the cells in 0.1 mol/L PBS for 3 times, the cells were incubated with 0.6% Triton X-100 in 0.1 mol/L PBS for 60 minutes at room temperature to permeabilize the cells. After washing the cells in 0.1 mol/L PBS for 3 times, the cells were blocked by 2% normal goat serum for 60 minutes at room temperature to block non-specific sites. The samples were incubated with

Fig. 1. Neurite outgrowth in different experimental conditions. Panel A: control; Panel B: gp120 (500 pmol/L); Panel C: ddC (50 μmol/L); Panel D: gp120 (500 pmol/L)+ddC (50 μmol/L); Panel E: gp120 (500 pmol/L)+IGF-1 (20 nmol/L); Panel F: ddC (50 μmol/L)+IGF-1 (20 nmol/L); Panel G: gp120 (500 pmol/L)+ddC (50 μmol/L)+IGF-1 (20 nmol/L). A2-G2 are enlargements of small neurons in A1-G1 with thin arrows. A3-G3 are enlargements of large neurons in A1-G1 with thick arrows. Scale bar=50 μm.
rabbit polyclonal anti-GAP-43 (1:500, abcam, Cambridge, MA, USA) overnight at 4°C, respectively. After washing the cells in 0.1 mol/L PBS 3 times, the samples were incubated by goat anti-rabbit conjugated to Cy3 (1:500, Abcam, Cambridge, MA, USA) for 45 minutes in the darkness. After washing the cells 3 times in 0.1 mol/L PBS, the cells were incubated with mouse monoclonal anti-MAP2 (1:400, Abcam, Cambridge, MA, USA) for 60 minutes in the dark. After washing the cells 3 times in 0.1 mol/L PBS, the cells were incubated with goat anti-mouse conjugated to Cy2 (1:200, Abcam, Cambridge, MA, USA) for 45 minutes in the darkness. After washing the cells 3 times in 0.1 mol/L PBS, the cover slips were placed on glass slides immediately (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and stored at 4°C until observation by fluorescent microscope.

Quantitative analysis of the percentage of GAP-43-expressing neurons

The MAP2-immunoreactive (IR) DRG neurons in five visual fields in the central part of each coverslip were counted as the positive neurons in each sample. All MAP2-IR DRG neurons were defined as small neurons (the diameter of neuronal cell body ≤25 μm) and large neurons (the diameter of neuronal cell body >25 μm). The number of GAP-43-IR neurons of small and large DRG neurons in the same visual field were also counted, respectively. Then the percentage of GAP-43-IR neurons of small and large DRG neurons could be obtained.

Statistical analysis

All experiments were performed in triplicate for each condition as one experiment. Five experiments (n=5) were finished for final analysis and reported as mean ± SD. All the data were processed for verifying normality test for Variable. If normality test failed, the data were analyzed with non-parametric test. If normality test passed, statistical analysis was evaluated with independent-sample t-test and analyzed using SPSS (version 13.0) software with p value<0.05 used to delineate significance for analysis of all results.

RESULTS

Total neurite length of small neurons in different experimental conditions

To determine the total neurite length of each small DRG neuron, DRG neuronal cultures at 72 hours after treatment with different agents were processed for fluorescent labeling of MAP2. The total neurite length of each small DRG neuron (the diameter of neuronal cell body ≤25 μm) was measured. The mean total neurite length of small neurons in control group, gp120 (500 pmol/L), ddC (50 μmol/L), gp120 (500 pmol/L)+ddC (50 μmol/L), gp120 (500 pmol/L)+IGF-1 (20 nmol/L), ddC (50 μmol/L)+IGF-1 (20 nmol/L), and gp120 (500 pmol/L)+ddC (50 μmol/L)+IGF-1 (20 nmol/L) treated cultures was 296.3 μm ± 19.5 μm, 205.5 μm ± 20.7 μm, 178.6 μm ± 15.9 μm, 167.6 μm ± 15.9 μm, 246.2 μm ± 19.1 μm, 222.7 μm ± 13.3 μm, and 205.6 μm ± 14.52 μm, respectively. Exposure of gp120 and/or ddC induced neurite retraction of small DRG neurons (gp120, F=0.014, p=0.000; ddC, F=0.420, p=0.000; gp120+ddC, F=0.301, p=0.000). Interestingly, ddC exposure alone caused more severe neurite retraction of small DRG neurons compared with that in gp120-treated cultures (F=0.172, p=0.047). The combination of gp120 and ddC did not induce any further decreases in total neurite length of small DRG neurons compared with that in ddC-treated cultures suggesting a ceiling effect. Exogenous IGF-1 application could partially reverse the neurite retraction of small DRG neurons (gp120, F=0.009, p=0.012; ddC, F=0.079, p=0.001; gp120+ddC, F=0.047, p=0.004) (Fig. 1, 2).

Total neurite length of large neurons in different experimental conditions

After treatment with different agents for 72 hours, the mean total neurite length of large neurons (the diameter of neuronal cell body >25 μm) was measured. The mean total neurite length of large neurons in control group, gp120 (500 pmol/L), ddC (50 μmol/L), gp120 (500 pmol/L)+ddC (50 μmol/L), gp120 (500 pmol/L)+IGF-1 (20 nmol/L), ddC (50 μmol/L)+IGF-1 (20 nmol/L), and gp120 (500 pmol/L)+ddC (50 μmol/L)+IGF-1 (20 nmol/L) treated cultures was 236.4 μm ± 15.1 μm, 122.0 μm ± 9.4 μm, 147.8 μm ± 16.1 μm, 113.2 μm ± 18.5 μm, 111.6 μm ± 15.1 μm, and 108.0 μm ± 14.3 μm, respectively. The mean total neurite length of large neurons in control group, gp120 (500 pmol/L), ddC (50 μmol/L), gp120 (500 pmol/L)+ddC (50 μmol/L), gp120 (500 pmol/L)+IGF-1 (20 nmol/L), ddC (50 μmol/L)+IGF-1 (20 nmol/L), and gp120 (500 pmol/L)+ddC (50 μmol/L)+IGF-1 (20 nmol/L) treated cultures was 236.4 μm ± 15.1 μm, 122.0 μm ± 9.4 μm, 147.8 μm ± 16.1 μm, 113.2 μm ± 18.5 μm, 111.6 μm ± 15.1 μm, and 108.0 μm ± 14.3 μm, respectively.

All experiments were performed in triplicate for each condition as one experiment. Five experiments (n=5) were finished for final analysis and reported as mean ± SD. All the data were processed for verifying normality test for Variable. If normality test failed, the data were analyzed with non-parametric test. If normality test passed, statistical analysis was evaluated with independent-sample t-test and analyzed using SPSS (version 13.0) software with p value<0.05 used to delineate significance for analysis of all results.

Fig. 2. Quantification of total neurite length of small neurons. Bar graphs with error bars represent mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

Fig. 3. Quantification of total neurite length of large neurons. Bar graphs with error bars represent mean ± SD. *p<0.05, **p<0.001.
Fig. 4. Double fluorescent labeling of MAP2 and GAP-43 of DRG neurons treatment with different agents. Panel A (control): A1, MAP2-IR neurons; A2, GAP-43-IR neurons; A3, overlay of A1 and A2. Panel B (500 pmol/L gp120): B1, MAP2-IR neurons; B2, GAP-43-IR neurons; B3, overlay of B1 and B2. Panel C (50 μmol/L ddC): C1, MAP2-IR neurons; C2, GAP-43-IR neurons; C3, overlay of C1 and C2. Panel D (500 pmol/L gp120+50 μmol/L ddC): D1, MAP2-IR neurons; D2, GAP-43-IR neurons; D3, overlay of D1 and D2. Panel E (500 pmol/L gp120+IGF-1): E1, MAP2-IR neurons; E2, GAP-43-IR neurons; E3, overlay of E1 and E2. Panel F (50 μmol/L ddC+IGF-1): F1, MAP2-IR neurons; F2, GAP-43-IR neurons; F3, overlay of F1 and F2. Panel G (500 pmol/L gp120+50 μmol/L ddC+IGF-1): G1, MAP2-IR neurons; G2, GAP-43-IR neurons; G3, overlay of G1 and G2. Scale bar=50 μm.
The percentage of GAP-43-IR neurons of small DRG neurons

To determine the percentage of GAP-43-IR neurons of small DRG neurons (the diameter of neuronal cell body ≤ 25 μm), DRG neuronal cultures at 72 hours after treatment with different agents were processed for double fluorescent labeling of MAP2 and GAP-43. The percentage of GAP-43-IR neurons of small DRG neurons in control group, gp120 (500 pmol/L), ddC (50 pmol/L)+ddC (50 pmol/L)+IGF-1 (20 nmol/L), gp120 (500 pmol/L)+IGF-1 (20 nmol/L), and gp120 (500 pmol/L)+IGF-1 (20 nmol/L) treated cultures was 39.6 ± 5.3%, 27.2 ± 3.4%, 20.6 ± 4.1%, 17.3 ± 3.9%, 34.0 ± 4.0%, 30.2 ± 4.2%, and 26.8 ± 4.0%, respectively. The percentage of GAP-43-IR neurons decreased significantly in gp120 and/or ddC treated cultures compared with that in ddC-treated cultures (F=0.012, p=0.026). The combination of gp120 and ddC did not cause any further decreases in the percentage of GAP-43-IR neurons of large DRG neurons compared with that in ddC-treated cultures (F=0.008, p=0.000). The percentage of GAP-43-IR neurons in large DRG neurons decreased significantly in gp120 and/or ddC treated cultures (gp120, F=0.002, p=0.000; ddC, F=0.003, p=0.008; gp120+ddC, F=0.371, p=0.000). gp120 caused more severe decreases in the percentage of GAP-43-IR neurons of large DRG neurons compared with that in gp120-treated cultures (Fig. 4, 6).

DISCUSSION

Treated with antiretroviral therapy, HIV-infected individuals are living longer but prone to the potentially neurotoxic effects of antiretroviral therapy (Gabbaï et al., 2013). Antiretroviral toxicity is an increasingly important issue in the management of HIV-infected patients (Rather et al., 2013). The neuropathic pain caused by DSP is very difficult to manage with few options available. The most common drugs used for neuropathic pain are usually not effective. The mechanisms responsible for peripheral neuropathy caused by HIV-infection itself or antiretroviral therapy are still not completely elucidated. It hence behooves us to explore the mechanisms and pursue new approaches to mitigate neurotoxicity or neurological complications.
caused by this peripheral neuropathy. Mounting evidence has implicated that IGF-1 plays an important role in protecting neuronal cells from injury (Li et al., 2013). The aim of the present study was to approach the questions of the neurotoxicity of the HIV-gp120 protein and the antiretroviral drug ddC on different subpopulations of DRG neurons and the neuroprotective effects of IGF-1 on specific subpopulations of DRG neurons with gp120- and/or ddC-induced neurotoxicity in vitro. The results showed that HIV-gp120 protein and/or antiretroviral drug ddC caused neurotoxicity of primary cultured DRG neurons. Interestingly, the severity of neurotoxicity induced by gp120 and ddC was different in different subpopulation of DRG neurons. gp120 mainly affected large diameter DRG neurons (>25 μm), whereas ddC mainly affected small diameter DRG neurons (≤25 μm). IGF-1 could reverse the neurotoxicity induced by gp120 and/or ddC on small, but not large, DRG neurons.

HIV envelope glycoprotein gp120 not only impairs adult neurogenesis and neurite outgrowth in the central nervous system (CNS) contributing to cognitive disorders (Lee et al., 2013), but also is highly related to HIV infection-related peripheral neuropathy (Smith, 2011). In the present study, gp120 mainly caused neurite retraction of large diameter DRG neurons with large fibers which are responsible for mechanical hypersensitivity. This finding is consistent with that HIV infection itself is linked to large fiber impairment (Kokotis et al., 2013).

Antiretroviral therapy has efficiently inhibited HIV replication and dramatically reduced mortality in HIV infection, but leads to a severe peripheral neuropathy which not only causes considerable disability and negatively affects quality of life, but also complicates all stages of the antiviral therapy. ddC is one of the most neurotoxic antiretroviral drugs on developing a neuropathy (Dalakas et al., 2001; Robinson et al., 2007). It has been observed that intraepidermal nerve fiber density in skin biopsies (ankle and thigh) decreased after neurotoxic compound ddC treatment (Kokotis et al., 2013). The decrease of intraepidermal nerve fiber density, which is a validated predictor of small unmyelinated nerve fiber damage (Myllyläki et al., 2012; Shikuma et al., 2012), appears to impair thin fiber conduction primarily. In the present study, ddC exposure mainly induced neurite retraction of small diameter DRG neurons with thin fibers which are responsible for pain hypersensitivity. This finding is consistent with that antiretroviral therapy primarily relates to thin fiber dysfunction (Kokotis et al., 2013).

IGF-1 is a basic peptide composed of 70 amino acids with a variety of functions in neuronal cells (Zheng and Quirion, 2006; Croci et al., 2011). IGF-1 plays an important role in promoting axonal growth from DRG neurons (Bomze et al., 2001; Jones et al., 2003; Seki et al., 2010) and supports to a population of predominantly nociceptive neurons in DRG which may contribute to neuropathic pain (Craner et al., 2002; Miura et al., 2011). Exogenous administration of IGF-1 after injury is neuroprotective and improves long-term neurological function (Guan, 2008). In the present study, IGF-1 application mainly reversed neurotoxicity of small diameter DRG neurons with ddC alone or combination treatment of gp120 and ddC. IGF-1 had less effects on gp120-induced neurotoxicity. These results suggested that IGF-1 could rescue small diameter DRG neurons with thin nociceptive fibers. The reason might be that IGF-1 and its receptor (IGF-IR) are expressed in small DRG neurons (Chirivella et al., 2012).

Side effects of antiretroviral therapy and HIV infection itself-induced neurological complications may overlap significantly (Suvada, 2013). It has been suggested that combination of perineural exposure to the HIV-gp120 protein and ddC treatment induced a more severe peripheral neuropathic pain than either HIV-gp120 protein or ddC treatment alone (Blackbeard et al., 2012). The advanced HIV infection-associated neuropathic pain is so severe that it is difficult to generalize about pain treatment. The neurotoxic antiretroviral drug burden considerably worsened the neuropathic pain symptoms of these patients who would give up the antiretroviral therapy which would aggravate HIV infection. The underlying complexities in managing pain symptoms in the population of advanced HIV disease require the neurobiologist and clinician to explore the mechanisms and implement new effective preventative or therapeutic strategies to minimize the negative impact on the patients’ quality of life. The results of our present study may reveal the novel mechanism which HIV-gp120 protein and ddC may target on different subpopulation of primary sensory DRG neurons for the neurotoxicity. The neurotrophic factor IGF-1 might be one of the potential principal factors on managing neuropathic pain symptoms of HIV infection- or antiretroviral therapy-related peripheral neuropathy.

In conclusion, both HIV-gp120 protein and/or antiretroviral drug ddC caused neurotoxicity of primary cultured DRG neurons and IGF-1 could partially reverse the neurotoxicity induced by gp120 and/or ddC. These findings provide evidence that HIV-gp120 protein and ddC may induce neurotoxicity on specific subset of primary sensory DRG neurons and may present a new therapeutic opportunity for managing HIV-associated neurotoxicity. The results of the present study offer new insights in elucidating the pathogenesis of HIV infection- or antiretroviral therapy-related peripheral neuropathy and facilitating the development of novel treatment strategies.

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REFERENCES

Acharjee, S., Zhu, Y., Maingat, F., Pardo, C., Ballanyi, K., Hollenberg, M. D. and Power, C. (2011) Proteinase-activated receptor-1 mediates dorsal root ganglion neuronal degeneration in HIV/AIDS. Brain 134 (Pt 11), 3209-3221.
Blackbeard, J., Wallace, V. C., O’Dea, K. P., Hasnie, F., Segerdahl, A., Pheby, T., Field, M. J., Takata, M. and Rice, A. S. (2012) The correlation between pain-related behaviour and spinal microglia in four distinct models of peripheral neuropathy. Eur. J. Pain 16, 1357-1367.
Bomze, H. M., Bulsara, K. R., Iskandar, B. J., Caroni, P. and Skene, J. H. (2001) Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons. Nat. Neurosci. 4, 38-43.
Chirivella, L., Cano-Jaimez, M., Pérez-Sánchez, F., Herrera, L., Carrero, J., Farríñas, I., Burks, D. J. and Kirstein, M. (2012) IRS2 signalling is required for the development of a subset of sensory spinal neurons. Eur. J. Neurosci. 35, 341-352.
Craner, M. J., Klein, J. P., Black, J. A. and Waxman, S. G. (2002) Preferential expression of IGF-1 in small DRG neurons and down-regulation following injury. Neuropathol. 13, 1649-1652.
Croci, L., Barili, V., Chia, D., Massimino, L., van Vugt, R., Masserdotti, G., Longhi, R., Rotwein, P. and Consalez, G. G. (2011) Local insulin-like growth factor I expression is essential for Purkinje neuron survival at birth. Cell Death Differ. 18, 48-59.
Dalakas, M. C., Semino-Mora, C. and Leon-Monzon, M. (2001) Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 3'-dideoxycytidine (ddC). *Lab. Invest.* 81, 1537-1544.

Gabbai, A. A., Castelo, A. and Oliveira, A. S. (2013) HIV peripheral neuropathy. *Handb. Clin. Neurol.* 115, 515-529.

Guán, J. (2008) Insulin-like growth factor-1 and its derivatives: potential pharmaceutical application for ischemic brain injury. *Recent Pat. CNS Drug Discov.* 3, 112-127.

Höschle, D. (2006) Cell culture models for the investigation of NRTI-induced mitochondrial toxicity: Relevance for the prediction of clinical toxicity. *Toxicol. In Vitro* 20, 535-546.

Huang, W., Calvo, M., Karu, K., Olausen, H. R., Bathgate, G., Okuse, K., Bennett, D. L. and Rice, A. S. (2013) A clinically relevant rodent model of the HIV antiretroviral drug stavudine induced painful peripheral neuropathy. *Pain* 154, 560-575.

Jones, D. M., Tucker, B. A., Rahimtula, M. and Mearrow, K. M. (2003) The synergistic effects of NGF and IGF-1 on neurite growth in adult sensory neurons: convergence on the PI 3-kinase signaling pathway. *J. Neurochem.* 86, 1116-1128.

Kokotis, P., Schmelz, M., Papadimas, G. K., Skopelitis, E. E., Aroni, K., Kordossis, T. and Karandreas, N. (2013) Polyneuropathy induced by HIV disease and antiretroviral therapy. *Clin. Neurophysiol.* 124, 176-182.

Lee, M. H., Amin, N. D., Venkatesan, A., Wang, T., Tyagi, R., Pant, H. C. and Nath, A. (2013) Impaired neurogenesis and neurite outgrowth in an HIV-gp120 transgenic model is reversed by exercise via BDNF production and Cdk5 regulation. *J. Neurovirol.* 19, 418-431.

Li, H., Dong, H., Li, J., Liu, H., Liu, Z. and Li, Z. (2013) Neuroprotective effect of insulin-like growth factor-1: Effects on tyrosine kinase receptor (Trk) expression in dorsal root ganglion neurons with glutamate-induced excitotoxicity in vitro. *Brain Res. Bull.* 97C, 86-95.

Liu, H., Lu, J., He, Y., Yuan, B., Li, Y. and Li, X. (2014) Insulin-like growth factor-1 prevents dorsal root ganglion neuronal tyrosine kinase receptor expression alterations induced by dideoxycytidine in vitro. *Cell. Mol. Neurobiol.* 34, 183-194.

Luma, H. N., Tchaleu, B. C., Doualla, M. S., Temfack, E., Sopouassi, V. N., Mapouré, Y. N. and Djentcheu, V. D. (2012) HIV-associated sensory neuropathy in HIV-1 infected patients at the Douala General Hospital in Cameroon: a cross-sectional study. *AIDS Res. Ther.* 9, 35.

Lunn, J. S., Pacut, C., Backus, C., Hong, Y., Johe, K., Hefferan, M., Marsala, M. and Feldman, E. L. (2010) The pleotropic effects of insulin-like growth factor-1 on human spinal cord neural progenitor cells. *Stem Cells Dev.* 19, 1983-1993.

Miura, M., Sasaki, M., Mizukoshi, K., Shibasaki, M., Izumi, Y., Shimosato, G., and Amaya, F. (2011) Peripheral sensitization caused by insulin-like growth factor 1 contributes to pain hypersensitivity after tissue injury. *Pain* 152, 888-895.

Myllymäki, M., Panoutopolou, I. G. and Särkkä, A. (2012) Analysis of spatial structure of epidermal nerve entry point patterns based on replicated data. *J. Microsc.* 247, 228-239.

Ng, K., Kumar, K., Brew, B. and Burke, D. (2011) Axonal excitability in viral polyneuropathy and nucleoside neuropathy in HIV patients. *J. Neurol. Neurosurg. Psychiatry* 82, 978-980.

Rather, Z. A., Chowta, M. N., Raju, G. J., Mubeen, F. (2013) Evaluation of the adverse reactions of antiretroviral drug regimens in a tertiary care hospital. *Indian J. Pharmacol.* 45, 145-148.

Robinson, B., Li, Z. and Nath, A. (2007) Nucleoside reverse transcriptase inhibitors and human immunodeficiency virus proteins cause axonal injury in human dorsal root ganglia cultures. *J. Neurovirol.* 13, 160-167.

Schütz, G. and Robinson-Papp, J. (2013) HIV-related neuropathy: current perspectives. *HIV AIDS* 5, 243-251.

Seki, T., Abdel Nazeer, A., Sekimoto, K., Guo, Y., Al-jahdari, W. and Saito, S. (2010) Fibroblast growth factor and insulin-like growth factor rescue growth cones of sensory neurites from collapse after tetracaine-induced injury. *Anesth. Analg.* 110, 1468-1472.

Shikuma, C., Gerschenson, M., Ananworanich, J., Valcour, V., Teeratakulpisarn, N., Jadwattanakul, T., Degruttola, V., Liang CY, McArthur, J., Ebenezer, G., Chomchey, N., Praihirunkit, P., Hongchookiath, P., Mathajitphun, P., Nakamoto, B., Hauer, P., Phanuphak, P. and Phanuphak, N. Search 003 protocol team. (2012) Determinants of epidermal nerve fibre density in antiretroviral-naive HIV-infected individuals. *HIV Med.* 13, 602-608.

Smith, H. S. (2011) Treatment considerations in painful HIV-related neuropathy. *Pain Physician* 14, E505-524.

Suvada, J. (2013) Neuropathic and neurocognitive complications of antiretroviral therapy among HIV-infected patients. *Neuro Endocrinol. Lett.* 34(Suppl1), 5-11.

Wood, T. L., Lولoladze, V., Alltzer, S., Gangoli, N., Levison, S. W., Brywe, K. G., Mallard, C. and Hagberg, H. (2007) Delayed IGF-1 administration rescues oligodendrocyte progenitors from glutamate-induced cell death and hypoxic-ischemic brain damage. *Dev. Neurosci.* 29, 302-310.

Zheng, W., Ouyang, H., Zheng, X., Liu, S., Mata, M., Fink, D. J. and Hao, S. (2011) Glial TNFα in the spinal cord regulates neuropathic pain induced by HIV gp120 application in rats. *Mol. Pain* 7, 40.

Zheng, W. H. and Quinon, R. (2006) Insulin-like growth factor-1 (IGF-1) induces the activation/phosphorylation of Akt kinase and cAMP response element-binding protein (CREB) by activating different signaling pathways in PC12 cells. *BMC Neurosci.* 7, 51.