Nerve Growth Factor Signaling Pathways Modulate HIV Vpr’s Actions on Sensory Neurons: A Potential Target for Treatment of Distal Sensory Polyneuropathy in HIV/AIDS

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Abstract

Over 35 million people are infected currently with the Human Immunodeficiency Virus (HIV), of whom 30-50% will experience Distal Sensory Polyneuropathy (DSP), usually causing paresthesiae and neuropathic pain, particularly in the feet. This presentation is identical to patients with Diabetic DSP. Current regimens for treating neuropathic pain have limited benefits. Thus, a deeper understanding of the mechanisms of HIV-DSP is imperative to permit the rational development of new therapies. Transgenic mice expressing the HIV-1 viral protein R (Vpr) show footpad epidermal denervation and allodynia as observed in HIV-infected patients. We found that exogenous Vpr inhibits axon outgrowth, causes hyperexcitability and increases cytosolic calcium in cultured dorsal root ganglion neurons (DRGN). Exposure of DRGN to nerve growth factor (NGF) or modulating NGF signaling pathways before Vpr treatment can block its effects. These findings will be extended to in vivo models to determine if altering the NGF signaling pathway can prevent Vpr-induced denervation and allodynia.

Keywords: Human Immunodeficiency Virus; Acquired Immunodeficiency Syndrome; Nerve Growth Factor; Dorsal root ganglion neurons; p75 neurotrophic receptor; Distal sensory polyneuropathy; Viral Protein R

Introduction

Distal sensory polyneuropathy (DSP) is the major peripheral nervous system disorder in 30-50% of people infected with Human Immunodeficiency Virus (HIV) showing Acquired Immunodeficiency Syndrome (AIDS). HIV-DSP symptoms include chronic neuropathic pain, alldynia, hyperalgesia, dysesthesia and gait dysfunction [1-10]. Current analogesics such as opioids, tricyclic antidepressants, anticonvulsants, capsaicin and topical anesthetics, show limited benefits as treatments for HIV-DSP and moreover, are often poorly tolerated [11-14]. Therefore, neuropathic pain associated with DSP can have devastating effects on the quality of life for affected patients, leading to depression and, at times, suicide. Importantly, current antiretroviral therapy regimes have little impact on HIV-DSP [8,15,16] and in fact older antiretroviral drugs (e.g. stavudine, zalcitabine, didanosine), actually worsened the signs and symptoms of DSP. HIV-1 encodes several accessory proteins including the 96 amino acid (14 kD) viral protein R (Vpr) [17], which is required for HIV infection of macrophages. Vpr expression in brain macrophages/microglia causes a neurodegenerative phenotype that resembles HIV-associated neurocognitive disorder (‘Neuro-AIDS’) [18]. The latter report indicated that impairment of CNS neurons is mediated by Vpr effects on ionconductance’s, thus altering their membrane potential while it also appears to initiate apoptosis by promoting caspase-3 and -9 activation. Herein we review Vpr’s involvement in DSP.

Possible sites and mechanisms of neuropathic pain in HIV-DSP

The lack of a targeted specific treatment of HIV-DSP is related to a limited understanding of both the primary anatomical site of injury and cellular mechanism(s) underlying DSP. The following comments are based on our recently published findings along with excellent reviews [5,6,10,19-21] as summarized in Figure 1. One of three candidate anatomical sites of injury is within the spinal cord where dorsal horn neurons might have developed long-term potentiation and/or disinhibition due to excessive or diminished synaptic inputs. Altered pathological inputs might originate from primary sensory dorsal root ganglion neuronal somas (DRGN) or their axons that might have been injured in the early stage of the infection. This central sensitization is transmitted to thalamic, reticular and then ultimately to cortical neural circuits to evoke pain sensation. A second candidate site lies in the extremities, particularly the feet where DSP-associated pain, numbness and paresthesia are typically perceived. Specifically, the site is within the skin of the feet at free nerve endings of distal axons (‘fibers’) that specifically sense pain (‘nocis’), mechano- or thermo-stimuli. This view is supported by data showing loss of epidermal free nerve endings in calf skin biopsies from affected patients with DSP [13]. Accordingly, chronic versus alldynic pain may be due to spontaneous action potential firing in injured distal axon endings of DRGN nociceptors versus mechano- or thermoreceptors, respectively. Finally, a third site of injury may be due to altered discharge within the DRGN soma located close to the spinal cord. These action potentials could propagate along the proximal (‘central’) axon to the dorsal horn via the same route as action potentials originating from the distal axon. It is therefore unknown whether such potential peripheral hyperexcitability in HIV-

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DSP pain manifests at the DRGN soma and/or distal axon and which specific DRGN classes are involved.

As stated above, experimental models suggest that Vpr appears to be causally involved in HIV-related damage of CNS neurons [18]. This finding prompted our group to study Vpr roles in peripheral processes involved in HIV-DSP as summarized in Figure 2 and 3. Two animal models were instrumental for our findings in that regard. Firstly, we developed transgenic vpr/RAG1−/− mice which, similar to HIV/AIDS patients, constitutively express Vpr while being immunodeficient due to the absence of mature B or T cell lymphocytes [22]. Secondly, we used cultured DRGN from neonatal and adult rats (as well as human fetal DRGNs) to study the effects of exogenous (recombinant) Vpr under defined in vitro conditions [22,23]. We found that vpr/RAG1−/− mice display epidermal denervation and allodynia (also seen in the lower extremities HIV-infected people) but not control mice, while the animal’s footpads show decreased expression of nerve growth factor signaling pathways.
Figure 2: Our published findings established that in vivo Vpr expression mimics HIV-DSP features in transgenic immunosuppressed vpr/RAG1-/- mice [22,23].

A) Von Frey filament testing confirmed vpr/RAG1-/- mice have a lower pain threshold than their RAG1-/- age-matched control mice. B) Epidermal nerve fiber counts indicated the vpr/RAG1-/- mice have significantly less free nerve terminals than the RAG1-/- age-matched control mice.

A. Vpr Causes Allodynia

B. Vpr Decreases Footpad Innervation

Figure 3: Our studies also showed that nerve growth factor (NGF) counteracts Vpr's effects by acting on TrkAR and p75ntr signaling. A) DRG neuronal cultures pre-treated with NGF or a p75 receptor antagonist were protected from the neurite-inhibiting effects of recombinant Vpr. B) p75 receptor antagonism blocks the Vpr-induced decrease in the neurite-promoting protein pGSK3β. C) Vpr immediately causes an increase in intracellular calcium levels in DRG neurons which is inhibited by pretreatment with p75 receptor antagonism.

A. NGF or p75 Antagonism Blocks Vpr-induced Decrease in Neurite Extension

B. NGF Blocks Vpr-induced Inhibition of Axon Promoting Protein Expression

C. Vpr increases DRG Excitability & Cytosolic Calcium Levels

It is interesting to note that HIV- and Diabetic DSP both display a denervation at the site of pain as well as a decreased NGF expression in the skin [23-25]. As it has been previously understood that NGF supports survival and skin innervation in adult animals and enhances regeneration of small diameter nociceptive DRGN [26-29], this indicated that NGF depletion might be involved in the pathogenesis of HIV-DSP. In support of this hypothesis, a clinical trial showed that NGF injection (0.1-0.3 µg/kg) into the feet of HIV/AIDS patients improved neuropathic pain symptoms [4]. However, the therapeutic potential of the approach was limited in that study by the occurrence of painful inflammation at the injection site. Based on these findings, we hypothesized that epidermal NGF depletion plays also a pivotal role in distal denervation of DRGN in our in vivo and in vitro animal models and thus studied the involvement of specific NGF receptors in Vpr-mediated HIV-DSP.

The nocice- and mechanoreceptive DRGN peripheral axons that reside in the skin, a source of NGF, express two NGF receptors, the high-affinity tyrosine kinase A receptor (TrkAR) and the low-affinity pan-specific p75 neurotrophin receptor (p75ntr) [27,28]. Previous studies on cultured DRGN have shown that NGF (0.1-100 ng/ml) binds to the TrkAR to phosphorylate glycogen synthase kinase β (pGSK3β) and activate the phosphoinositol-3 kinase signaling cascade for promoting axon outgrowth [18,27]. In contrast, at higher doses (100-1000 ng/ml), NGF binds to the p75ntr to inhibit axon outgrowth in DRGN [30]. In
line with these observations, we showed in cultured rat DRGN that recombinant Vpr (100 nM) decreases expression of both TrkA receptor and pGSK3β which hampers neurite extension and increases their membrane excitability with a concomitant rise of cytosolic calcium [23]. Moreover, we demonstrated in this report that pre-exposure of DRGN to NGF (50 ng/ml) negates these Vpr-mediated effects, thus uncovering a potential target for a rational pharmacological HIV-DSP therapy. In that regard, we also found in that study that both blocking the p75NTR with the functional antibody REX and activating TrkA in vivo with a TrkA agonist inhibits Vpr-mediated inhibition of neurite growth similar to application of NGF.

These exciting findings suggest a possible molecular means by which Vpr affects TrkA/p75 signaling, however the mechanism is not yet known. It is possible that Vpr directly or indirectly binds to one or both the receptors, affecting their internalization and or their intracellular signaling. Our calcium imaging data, however clearly shows that NGF specifically inhibits the Vpr-induced increase in intracellular calcium, strongly suggesting a direct means for NGF to block the Vpr-induced affects in vitro [23]. Thus we do not believe that Vpr and NGF merely compete for pro-apoptotic and pro-survival pathways, respectively.

Where do we go from here?

The above study involving in vitro neurite growth, molecular signaling and Ca²⁺ imaging analyses indicated two potential pharmacological strategies for designing a specific treatment for HIV-DSP, i.e. by activating the TrkA and/or inhibiting the p75NTR (or their associated signaling pathways). While systemic application of a successful novel drug may treat the disease, it is still important to identify the primary site of its action. Research on HIV-DSP using DRGN cultures, including our above studies of Vpr effects, has allowed for dose response curve determination of HIV-DSP-related neurotoxic agents and testing of candidate therapeutic drugs. Such tests were mostly based on patch-clamp recording from the DRGN somata or live-cell imaging to monitor how the drugs influence the axonal outgrowth peripheral sensory neurons and their viability evidenced e.g. via fluorescence imaging of cytosolic calcium or mitochondrial potential dynamics. Certainly, data from DRGN cultures are pivotal for the understanding of molecular signaling pathways in HIV-DSP and other types of neuropathic pain. However, cultures have also limitations regarding identifying the primary site from which pain in HIV-DSP originates. Specifically: (i) modality-specific sensitivity of affected DRGN somata and/or axons, as in the intact animal, cannot be determined in vitro, (ii) ion channel dysfunction in cultured DRGN, mostly obtained from fetal or neonatal animals, may differ from that in adult cells in vivo, (iii) culture conditions may affect ion channel expression, (iv) cellular processes cannot be identified as dendrites versus proximal or distal axons and, consequently (v) patch-clamp recording does not delineate if affected ion channels are located on the DRGN soma, dendrites or (distal) axon.

To overcome these limitations, we propose to focus future efforts on identifying in vpr/RAG1−/− mice [22] both the primary site and ionic mechanism in the proposed DRGN/axon hyperexcitability. Specific affected DRGN classes can be identified either in anesthetized or decerebrated vpr/RAG1−/− mice in vivo via adequate footpad sensory stimulation combined with recording of their extracellular action potential discharge. For these studies, we will perform a laminectomy of the lumbar Spinal cord to enable compound or single fiber action potential recording with hook electrodes or, alternatively, with fine tungsten electrodes for microneurography of the DRG axons [6,31-35]. We expect that such analyses will reveal that nociceptive DRGN/axons are spontaneously active and their activation threshold is lower in vpr/RAG1−/− mice compared to control mice. Moreover, alloydynia in vpr/RAG1−/− mice might be related to ectopic discharge in (normally ‘silent’) C fibers and possibly also in mechanosensitive Aδ or thinly myelinated Aβ-pain fibers. If such axons are spontaneously active in vivo/RAG1−/− mice, this might be indicative of paresthesia [36]. It is also possible that the action potential threshold in thermosensitive axons is changed in vpr/RAG1−/− mice and that they show spontaneous and/or ectopic firing. Underlying (ion channel) dysfunctions can be studied using either intracellular microelectrode or patch-clamp recording from DRGN somata in the functionally intact preparations or by microneurography and threshold tracking in the distal axons [6,31,33-35,37].

References


