Gene therapy with HSV encoding p55TNFR gene for HIV neuropathic pain: an evidence-based mini-review

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Abstract
While effective antiretroviral treatment makes human immunodeficiency virus (HIV)-related death decreased dramatically, neuropathic pain becomes one of the most common complications in patients with HIV/acquired immunodeficiency syndrome (AIDS). The exact mechanisms of HIV-related neuropathic pain are not well understood yet, and no effective therapy is for HIV-pain. Evidence has shown that proinflammatory factors (e.g., tumor necrosis factor alpha (TNFα)) released from glia, are critical to contributing to chronic pain. Preclinical studies have demonstrated that non-replicating herpes simplex virus (HSV)-based vector expressing human enkephalin reduces inflammatory pain, neuropathic pain, or cancer pain in animal models. In this review, we describe recent advances in the use of HSV-based gene transfer for the treatment of HIV pain, with a special focus on the use of HSV-mediated soluble TNF receptor 1 (neutralizing TNFα in function) in HIV neuropathic pain model.

Keywords
HIV, Neuropathic pain, Soluble TNF receptor, and Gene therapy

Introduction
The United States Centers for Disease Control reports that an estimated 1.1 million people were living with human immunodeficiency virus (HIV) at the end of 2014 and that 39,513 people in 2015 were diagnosed with HIV infection in the United States (https://www.cdc.gov/hiv/basics/statistics.html, last date accessed June 24, 2017). Although effective antiretroviral therapy (ART) makes HIV become a treatable, chronic disease [1,2], new challenges are emerging in managing HIV. Chronic pain becomes one of the most common complications in patients with HIV/acquired immunodeficiency syndrome (AIDS). HIV-related pain is often underestimated in HIV/AIDS patients while the main focus is on immunosuppression and opportunistic infections. HIV neuropathic pain (HIV-NP) is refractory, and the current available chronic pain therapies are not effective to HIV-NP. This article reviews current researches from our work and others focusing on the pathophysiological mechanisms of HIV-neuropathic pain and gene therapy.

HIV-Related Neuropathic Pain
HIV sensory neuropathies contain distal sensory polyneuropathy as results of both HIV infection and antiretroviral drug-induced toxic neuropathies [3-6]. Clinical characteristics of distal sensory polyneuropathy and ART-induced toxic neuropathies are very similar. Neurotoxic ART has even been removed from pharmacies entirely in developed countries. Evidence shows that many people with HIV alive today, have ever been on numerous therapeutic regimens with neurotoxic drugs, and that they have already developed persistent painful neuropathy [7,8]. HIV-NP is typically bilateral, of gradual onset, and described as ‘aching’, ‘painful numbness’, or ‘burning’ [9]. Pathological feature of HIV-NP includes loss of sensory neurons of the dorsal root ganglion (DRG), Wallerian degeneration of the long axons in distal area, infiltration of macrophage into the DRG, and a ‘dying back’ sensory neuropathy [10-14]. However, the precise mechanisms of HIV-NP remain unknown yet and no effective therapy for HIV-NP.

Proinflammatory factors
Early studies have demonstrated that glia infected/activated by HIV release proinflammatory factors, such as tumor necrosis factor alpha (TNFα) and interleukin 1 (IL-1) [15]. Infiltration of inflammatory lymphocyte and macrophage to the DRG of AIDS patients produces pro-inflammatory cytokines including TNFα [12,16-20]. There is an increased TNFα in human CSF [21-25] and brain tissue [25-28] in patients with HIV. An interaction of TNFα and HIV infection enhances toxic chemokine products [29,30]. It is known that proinflammatory cytokines play an important role in the development and maintenance of neuropathic pain [31-35]. Proinflammatory mediators are critical to enhancing HIV-NP [36]. Intrathecal administration of gp120 induces acute pain and spinal proinflammatory cytokine release [37]. Pe-
Peripheral gp120 increases TNFα within the nerve trunk [38], intense glial activation in the spinal cord in parallel with neuropathic pain behaviors [38]. We have reported that peripheral gp120 application onto the rat sciatic nerve upregulates TNFα in the L4/5 DRGs and spinal cord [39]. Systemic 2′,3′-dideoxycytidine (ddC), one drug of ART lowers mechanical threshold [40,41] and increases both mRNA and protein of TNFα in the spinal cord dorsal horn (SCDH) [41]. Inhibition of TNFα or soluble TNF receptor reduces mechanical allodynia induced by gp120 application [41].

Therefore, it is possible that TNFα signal is involved in the induction and/or progression of HIV-NP.

**Reactive oxygen species and C/EBPβ in HIV**

Oxidative stress evokes many signaling events [42]. Mitochondria are the main source of reactive oxygen species (ROS). ROS plays a role in different pain models [43-48]. ROS scavengers produce a strong antinociceptive effect in persistent pain models [49]. Oxidative stress is involved in the pathogenesis of neuroAIDS [50]. HIV infection and ART can evoke rapid neurotoxicity [51]. Either HIV gp120 or ddC plays a role in initiation and/or intensification of ROS [52,53]. Intrathecal gp120 induces spinal release of nitric oxide (NO) as well as proinflammatory cytokines; pretreatment with NO synthase (NOS) inhibitor abolishes gp120-induced mechanical allodynia [54]. Importantly, ROS evoked by HIV infection, induces apoptosis through TNFα and its receptors [52]. Mitochondrial DNA (mtDNA) is critical for oxidative phosphorylation complex I proteins. DNA poly-merase-γ is important for replication of mtDNA. ARTs inhibit Poly-merase-γ, resulting in mitochondrial respiratory chain dysfunction and oxidative phosphorylation deficits [51]. Systemic ddC induces neuropathic pain and lowers the activity of endogenous manganese superoxide dismutase (SOD2) in the SCDH; ROS scavengers significantly reduce mechanical allodynia [55].

CCAAT/enhancer binding proteins (C/EBPs) are transcriptional factors in cell development and induction of inflammatory factors in the peripheral and central nervous system [56]. C/EBPβ plays an important role in a variety of HIV disease stages [57]. An increase in C/EBPβ mRNA is found in the brain tissue of HIV-1 encephalitis patients [58]. We have found that combination of peripheral gp120 with systemic ddC increases pC/EBPβ in the SCDH [59], suggesting that pC/EBPβ plays a role in HIV-NP.

**HSV Vector for Gene Therapy of Neuropathic Pain**

During natural infection of herpes simplex virus (HSV), HSV is carried by retrograde axonal transport from the site of original inoculation to the neuronal perikaryon. Latently infected neurons function normally and are not rejected by the host immune response (see review [60,61]). HSV-1 genome is a linear double-stranded DNA, and has more than 75 genes coded in the 152 kb genome [60,61]. HSV genes are expressed in a well-ordered temporal cascade of immediate early (IE) genes, followed by early genes, and subsequently late gene products; both early genes and the late genes require synthesis of IE gene products [60,61]. Deleting essential IE genes from the HSV genome makes it non-replicating recombinant [62], but the virus are still able to be used to effectively deliver target gene products [63,64]. Gene transfer mediated by HSV vector may provide a promising approach to the management of neuropathic pain. HSV vector encoding human preproenkephalin gene after transduction of DRG neurons by hindpaw injection [65], produces an antinociceptive effect in different pain models [66-68]. We have reported that HSV vectors expressing enkephalin, p55 TNF soluble receptor (p55TNFSR), interleukin-10, and interleukin-4 produce antinociceptive effects in preclinical pain models [69-75]. Fink and colleagues reported phase 1 clinical trial using HSV vector encoding human preproenkephalin in patients with cancer pain [76]. The clinical trial assessed the safety and explored the potential efficacy of this approach in humans, indicating that it may be effective in reducing cancer pain [77].

The distribution of systemically administered drugs to the brain may be limited by the blood-brain barrier [65], and they produce systemic side effects. Gene transfer that permanently release gene products, might be a useful alternative to regular pharmacological approaches [65]. Gene transfer of HSV vector may represent a platform technology---nerve targeting drug delivery system [77]. Viral vectors, however, show toxicity and inflammation from ‘leaky’ expression of viral genes and reaction to the vector coat protein in pre-immune animals [65,78]. Despite these limitations, ours and other studies have shown that HSV vector is still a highly effective gene delivery approach to treating peripheral and central nervous diseases [65,79,80].

**TNFSR mediated by HSV vector produces antiallo-odynamic effect in HIV-NP**

Our report has shown that the HIV gp120 application onto the sciatic nerve induces upregulation of TNFα, C-X-C chemokine receptor type 4 (CXCR4, a co-receptor of HIV), stromal cell-derived factor 1-α (SDF1-α, CXCR4 ligand) in both the DRG and the lumbar spinal dorsal horn [81]. Soluble TNF receptor (TNFSR) blocks bioactivity of TNFα. HSV vector encoding p55TNFSR gene (T0TNFSR) reduces mechanical allodynia and lowers TNFα, CXCR4 and SDF1-α induced by gp120 in the DRG and SCDH [81], suggesting that the pathway of TNFα to the CXCR4/SDF1 has an important role in the HIV-NP and that inhibiting proinflammatory cytokines/chemokines reduce...
neuropathic pain. In another model of HIV-NP induced by intraperitoneal ddC [40], ddC induces upregulation of TNFα, SDF1-α, and CXCR4 in both the lumbar spinal cord and the L4/5 DRG; T0TNFSR reduced mechanical allodynia and suppressed TNFα, SDF1-α, and CXCR4 in the lumbar SCDH and DRG [82], indicating that TNFα is

![Graph](image1.png)

**Figure 1:** Antinociceptive effect of p55TNFRI mediated by HSV vectors. Mechanical sensitivity was examined through the measurement of foot withdrawal frequencies to a sequential series of calibrated von Frey filaments applied in ascending order to the plantar surface of the foot (98). HSV vector T0TNFSR or T0Z was inoculated into the hindpaws 1 week post gp120/ddC. The occurrence of foot withdrawal for each trial was expressed as a percentage response frequency. Two weeks after HSV vectors, foot withdrawal frequencies to calibrated von Frey filaments in rats with subcutaneous inoculation of T0TNFSR were significantly lower than that in T0Z at filaments of 3.6, 5.5, 8.5, and 11.8 gram, * P<0.05, *** P<0.001 vs. T0TNFSR, t test, n=6.

![Graph](image2.png)

**Figure 2:** The expression of p55TNFRI mediated by the HSV vectors. One week after gp120/ddC, T0TNFSR or T0Z was inoculated into the hindpaws. On day 14 post gp120/ddC vector, the L4/5 dorsal root ganglion (DRG) was harvested, and western blot assays were conducted for testing TNFRI. T0TNFSR injection significantly induced the expression of TNFRI compared with T0Z in the L4/5 DRG, ** P<0.01 vs. T0Z, t test, n= 6.

![Images](image3.png)

**Figure 3:** The effect of p55TNFRI mediated by the HSV vectors on mitochondrial superoxide in the SCDH at 2 weeks post HSV vectors. One week post gp120/ddC, neuropathic rats received hindpaw injection of T0TNFSR or T0Z into ipsilateral hindpaw of rats. Two weeks after HSV vector, MitoSox Red was intrathecally injected 70 min prior to perfusion. The representative image of MitoSox red for mitochondrial superoxide in sham+T0Z, gp120/ddC+T0Z, and gp120/ddC+T0TNFSR, was shown in Figure A, B, and C, respectively, scale bar, 50µm. (D) The number of mitochondrial superoxide positive cells in the SCDH lamina I-II and III-V was shown, ** P<0.01, *** P<0.001, one way ANOVA with post hoc PLSD test, mean ± SEM, n=5-6 rats.
involved in the ARTs-related pain through the SDF1-α/CXCR4 system.

We have reported that combination of peripheral gp120 with systemic ddC (gp120/ddC) lowers mechanical threshold for more than 3 weeks, and that the minimum of mechanical threshold occurs around 2 weeks after gp120/ddC [59,83,84]. Previous studies show that HSV vector T0TNFSR reduces neuropathic pain induced by spinal nerve injury [69]. In gp120/ddC model, 2 weeks post HSV vector, T0TNFSR significantly reduced foot withdrawal frequencies (Figure 3D), and increased the expression of soluble TNFRI in the L4/5 DRG (Figure 2).

TNFSR mediated by the HSV vector reduces mitochondrial superoxide in gp120/ddC model

Oxidative stress causes many signaling events [42]. HIV gp120 or ddC induces ROS [52,53]. HIV gp120 application onto the sciatic nerve upregulates spinal mitochondrial superoxide [73,85]. We reported that gp120/ddC increased spinal mitochondrial superoxide [59,84] using MitoSox positive cell imaging (a marker of mitochondrial superoxide) [86,87]. Figure 3A-C showed the representative MitoSox positive cell imaging in the gp120/ddC model. The increased number of MitoSox positive cells in the gp120/ddC model was decreased by HSV vector T0TNFSR (Figure 3D), suggesting that TNFSR suppresses neuropathic pain through reducing spinal ROS.

TNFSR mediated by the HSV vector inhibits pC/EBPβ in the gp120/ddC neuropathic pain model

C/EBP plays a role in induction of inflammatory mediators in CNS [56]. HIV patients show upregulation of C/EBPβ mRNA in the brain tissue [58]. We have shown that HIV-NP increases phosphorylation of C/EBPβ (pC/EBPβ) [59]. Figure 4A-C revealed the representative pC/EBPβ-IR images in the gp120/ddC model. Treatment with gp120/ddC increased pC/EBPβ-IR expression; the upregulated pC/EBPβ-IR was suppressed by T0TNFSR (Figure 4D), suggesting that TNFSR reduces neuropathic pain through decreasing spinal pC/EBPβ.

The relationship of TNFα/TNF receptor activity and ROS or C/EBPβ in HIV-NP is still not clear. HIV gp120/ddC induces release of TNFα [83]. Through TNF receptor, TNFα triggers a cascade of events [88]. TNFα activates NMDA

Figure 4: The effect of p55TNFSR mediated by the HSV vectors on pC/EBPβ in the SDH at 2 weeks post HSV vectors. One week post gp120/ddC, neuropathic rats received injection of T0TNFSR or T0Z into ipsilateral hindpaw of rats. Two weeks after HSV vector, spinal pC/EBPβ-immunoreactivity (pC/EBPβ-IR) was examined using immunohistochemistry. The representative images of pC/EBPβ-IR in sham+T0Z, gp120/ddC+T0Z, and gp120/ddC+T0TNFSR were shown in Figure A, B, and C, respectively, scale bar, 50μm. (D) The quantitative signals of pC/EBPβ-IR in the SCDH were shown, ** P < 0.01, one way ANOVA with post hoc PLSD test, mean ± SEM, n=6 rats.
receptors to increase Ca^{2+} influx [89]. Indeed, HIV gp120 increases intracellular free Ca^{2+} concentration in the mouse SCDH cells [90]. ddC also increases spinal cytosolic Ca^{2+} concentration in painful neuropathy [91]. There is an interplay between cytosolic Ca^{2+} and mitochondrial ROS [92-94]. Spinal pCREB may make a contribution to the development of chronic pain [95]. Cytosolic Ca^{2+} may induce transcriptional factor CREB regulating C/EBPβ activity [96]. CREB binds C/EBPβ gene promoter, inducing the endogenous C/EBPβ expression [97]. Therefore, it is possible that TNFα-TNFR induces ROS, or pCREB/ C/EBPβ in HIV-NP, which need to be examined in the near future.

In summary, glia infected or activated after HIV release proinflammatory factors, such as TNFα. TNFα-TNF receptor signal may induce ROS or C/EBPβ in HIV-NP through complex pathway in the model of HIV-NP. Gene transfer using the HSV vector encoding the gene of TNF soluble receptor reduced neuropathic pain in animal studies, providing additional potential approach for successful treatment of HIV neuropathic pain.

**Conflict of Interest**

All authors declare no conflict of interest in the work.

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