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Viral Vector-based Gene Transfer for Treatment of Chronic Pain

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Introduction

Acute pain is a normal physiologic adaptive response to noxious stimuli that is essential for survival and maintenance of the integrity of the organism. Chronic pain, on the other hand, is a maladaptive, pathologic and persistent condition that results in a marked decrease in the quality of life and secondary symptoms such as anxiety and depression. Chronic pain is a common phenomenon. It is estimated that more than 50 million people in the United States alone are affected by chronic pain. Much of currently available clinic treatments for chronic pain are only partially effective and many are limited by off-target side effects, or have the potential of abuse.¹ Furthermore, analgesics that are highly effective in the management of pain often provide only very short-term relief, and some forms of pain, for example, neuropathic pain are practically difficult to treat.² In recent years, significant progress has been made in the fundamental understanding of the neurobiology of pain. In parallel, detailed molecular biologic characteristics of many of the receptors, neurotransmitters, neuropeptides, cytokines, chemokines, and ion channels that play significant roles in nociception have become available.^{3,4} Taking advantage of these advances, therapeutic gene transfer is emerging as an intriguing option for the development of novel treatment for chronic pain.

Gene therapy was first proposed as a treatment for genetically determined inherited disorders⁵ and the first successful human gene therapy for X-linked severe combined immunodeficiency in children, was reported 6 years ago.⁶ Therapeutic gene transfer for treatment of chronic pain is not directed at correcting a genetic defect. Rather the regional specialization of spinal cord and/or brain function and the widespread and redundant use of a limited repertoire of neurotransmitters and receptors in diverse pathways in the nervous system means that the local production of neurotransmitters achieved by therapeutic gene transfer may be used to achieve desired outcomes while avoiding unwanted adverse side effects that would result from activation of the same receptors in other pathways by a systemically administered drug.⁷ In this review we summarize recent studies of recombinant virus vectors for in vivo pain treatment in animal.

Viruses have the ability to infect and transduce their genome into host cells, and viral vector-based gene therapy takes advantage of the natural biology of virus to transduce exogenous genes into host cells. The general principle for creating a viral vector involves the deletion of nonessential genes from the virus, incapacitating its replication and, therefore, rendering it nonpathogenic, while retaining the structural motifs that allows the vector attach and transfer its genome into the host. Recently developed viral vectors provide a powerful vehicle for effective gene transfer to the nervous system.⁸ An ideal vector for gene therapy should achieve stable, tissue-specific regulated gene expression without eliciting the host immune response.⁹ The vector must possess sufficient capacity to carry the transgene, efficiently deliver that gene to the cell of interest, and express appropriate amounts of transgene product to provide a

therapeutic effect, all without detectable toxicity resulting from the vector itself.¹⁰ It should be noted that rendering a virus replication deficient does not assure elimination of direct cytotoxicity or abolition of host immune response caused by expression of other viral proteins not critical for replication.¹¹ The most commonly used viral vectors in studies of treatment of pain are based on recombinant adenovirus, adeno-associated virus (AAV), or herpes simplex virus. We will review the published data for each of these vectors below.

Adenovirus Vector

The adenovirus genome is composed of linear, double-stranded DNA of approximately 36 kb. Adenovirus types 2 and 5 (Ad 2 and Ad 5, respectively) have been developed for use in gene therapy.¹² Modification of the adenoviral genome by deletion of the region coding for early gene 1 (E1) creates a replication impaired vector providing sufficient space for gene insertions because E1 gene is essential for adenoviral replication. The natural biology of adenovirus is well understood, and tools for constructing a recombinant vectors are readily available.^{3,13} Adenovirus can be raised to high titers although the requirement of a helper virus, the vector functions independently of cell replication, is apparently nononcogenic, and has been used without complications in vaccines.¹⁴ Adenoviral DNA does not integrate into the host genome, which removes the complication of interrupting an important host gene, but may limit the vector ability to confer long-term expression.¹⁵

Finegold et al¹⁶ were the first to demonstrate that adenovirus-based vector could be used to transfer genes to the meninges surrounding the spinal cord, achieving an in vivo gene transfer paradigm for treating chronic pain in animal. The therapeutic vector consisted of a recombinant adenovirus encoding a secreted form of the potent endogenous opioid β -endorphin. The vector was tested in a model of persistent pain induced by subcutaneous injection of carrageenan into the plantar surface of 1 hindpaw in rats. Administration of the vector into the cerebrospinal fluid surrounding the spinal cord transduced meningeal pia mater cells. The resulting increase in β -endorphin secretion attenuated thermal hyperalgesia, yet had no effect on basal nociceptive responses.¹⁶

Spinal cord glia are critical to the establishment and maintenance of chronic pain through the release of proinflammatory cytokines, prostaglandin E2, and nitric oxide.⁴ Interleukin (IL)-10, an anti-inflammatory cytokine is known to suppress the production of proinflammatory cytokines.^{17,18} Acute intrathecal administration of rat IL-10 protein itself briefly reverses sciatic chronic constriction injury (CCI)-induced mechanical allodynia and thermal hyperalgesia.^{17,18} Watkins and coworkers demonstrated that intrathecal administration of adenoviral vector encoding for IL-10 injected over lumbosacral spinal cord led to elevated lumbosacral cerebrospinal fluid levels of IL-10, prevented sciatic nerve inflammation [sciatic inflammatory neuropathy (SIN)]-induced mechanical allodynia and attenuated chronic SIN-induced territorial, extraterritorial, and mirror-imaging mechanical allodynia.¹⁸ In addition, intrathecal administration of the adenovirus expressing IL-10 prevented intrathecal HIV-1 gp 120-induced mechanical allodynia, without affecting basal responses to thermal or mechanical stimuli.¹⁸ IL-2 is an important immuno-regulatory molecule in the central nervous system (CNS).^{19,20} IL-2 and IL-2 receptors are widely distributed in rat CNS²¹ and evidence showed that IL-2 had an antinociceptive effect in both central and peripheral nervous system²² and inhibited nociceptive responses of spinal dorsal horn neurons.²³ Yao et al²⁴ reported that intrathecal delivery of an adenoviral vector expressing IL-2 produced antinociceptive effects measured by basal nociceptive response and in a model of chronic neuropathic pain induced by sciatic nerve injury.

AAV Vector

AAV is a single-strand, nonenveloped DNA virus with a 4.7-kb genome.²⁵ There are 5 serotypes of human AAV and the AAV type 2 is used most commonly for vector development.²⁶ The AAV type 2 receptor is a membrane-associated heparin sulfate proteoglycan which is present in many cell surfaces, resulting in a broad host infectivity of this virus.²⁷ The AAV contains 2 genes, *rep* and *cap*, encoding for polypeptides essential for the replication and encapsulation of AAV. The 2 genes are flanked by viral inverted terminal repeats. AAV has low immunogenicity, which is important for the application of AAV vectors in human gene therapy. AAV integrates in the host genome and is transmitted to the progeny of the cells. The virus is incapable of autonomous replication and spread as a productive infection requires coinfection by a non-AAV helper virus (adenovirus or herpes virus). In recombinant AAV vectors, the viral *rep* and *cap* genes are replaced by a transgene cassette carrying promoter, transgene, and pA-site.¹² It has been shown that recombinant AAV vectors are suitable for in vitro and in vivo gene transfer into muscle, brain, hematopoietic progenitor cells, neurons, photoreceptor cells, and liver cells.^{12,28}

The spinal cord dorsal horn is the initial site of integration for ascending nociceptive neurotransmitters with descending modulation on pain.²⁹ Central sensitization characteristic of chronic pain is a condition of increased synaptic efficacy in which the recruitment of normally subthreshold inputs leads to the spread of pain sensitivity beyond the site of injury (secondary hyperalgesia) and the generation of pain in response to low-threshold inputs (allodynia).²⁹ The *N*-methyl-D-aspartate (NMDA) receptor is a major transducer of excitatory neurotransmission.³⁰ South et al³¹ demonstrated that in transgenic mice with a floxed NMDA receptor 1 (NR1), NR1 subunit was selectively deleted in the lumbar spinal cord of adult mice by a spinal dorsal horn injection of an AAV expressing Cre recombinase. NR1 subunit mRNA and dendritic protein were reduced by 80% in the area of the virus injection, and NMDA currents, but not α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid currents, were reduced 86% to 88% in lamina II neurons.³¹ The vector-mediated NR1 knock-out did not alter heat or cold paw-withdrawal latencies, mechanical threshold, or motor function, but injury-induced pain produced by intraplantar formalin was reduced by 70%.³¹

Huang and coworkers demonstrated that a recombinant AAV vector containing the cDNA for the μ -opioid receptor (μ -OR) injected into primary afferent neurons in dorsal root ganglia (DRG) of rats resulted in a long-lasting (>6 mo) increase in μ -OR expression in DRG neurons, that greatly potentiated the antinociceptive effects of morphine.^{32,33} Perforated patch recording indicated that the efficacy and potency of opioid inhibition of voltage-dependent Ca^{++} channels was enhanced in infected neurons.³³

Brain-derived neurotrophic factor (BDNF) is a potent survival and differentiation factor for many developing and injured neuronal systems³⁴ including CNS GABAergic³⁵ and serotonergic neurons.³⁶ Because of major role played by descending modulatory serotonergic fibers and the dorsal horn inhibitory GABAergic neurons, Eaton et al³⁷ reported that a recombinant AAV vector coding for BDNF intraparenchymally injected into spinal dorsal horn 1 week after sciatic CCI reversed allodynia and hyperalgesia induced by CCI beginning 1 week after vector injection. These data suggest that chronic neuropathic pain is sensitive to early spinal BDNF levels after partial nerve injury and that AAV-mediated gene transfer could potentially be used to reverse chronic pain after nervous injury.³⁷ Milligan et al³⁸ reported that intrathecal administration of AAV encoding IL-10 prevented and reversed neuropathic pain induced by CCI and SIN suggesting that intrathecal gene therapy to drive the production of IL-10 may prove to be an efficacious treatment for neuropathic pain.

The postsynaptic density proteins Shank and Homer cooperate to induce the maturation and enlargement of dendritic spines.³⁹ Homer1a is an activity-inducible short-splice variant of Homer that lacks dimerization capacity. Homer1a overexpression attenuates mGluR-evoked intracellular calcium release, probably by interfering with Homer-mediated coupling between type-I mGluRs and IP3 receptors. Homer1a inhibits growth of synapses and synaptic transmission.⁴¹ Glutamatergic signaling and intracellular calcium mobilization in the spinal cord are crucial for the development of nociceptive plasticity, which is associated with chronic pathologic pain.²⁹ Homer1a operates in a negative feedback loop to regulate the excitability of the pain pathway in an activity-dependent manner; Homer1a strongly attenuates calcium mobilization and MAP kinase activation induced by glutamate receptors and reduces synaptic contacts on spinal cord neurons that process pain inputs.⁴² Tappe et al⁴² demonstrated that AAV1/2 vectors coding for Homer1a intraparenchymally injected into the spinal dorsal horns of bilateral L3-5 segments showed a statistically significant reduction in the behavioral response and spinal phosphorylated extracellular signal-regulated kinase 1/2 MAP (pERK1/2) immunoreactivity to intraplantar formalin, and decrease in thermal hyperalgesia induced by complete Freund adjuvant.

Herpes Simplex Virus Vector

Herpes simplex virus (HSV) is an enveloped double-stranded 152-kb DNA virus; the mature wild-type virion consists of a core of DNA surrounded by an icosahedral capsid.⁴³ Immediately after entry of the viral DNA into the nucleus, expression of 5 viral immediate-early (IE) genes proceeding in the absence of de novo viral protein synthesis commences.⁴⁴ Expression of the IE gene products initiates a cascade of viral gene expression, beginning with transcription of early genes that primarily encode enzymes involved in DNA replication followed by late genes, that primarily encoding structural components of the virion.⁴⁶ In nature wild-type HSV is spread by contact, infecting epithelial cells of the skin or mucous membranes with viruses released at the site of the primary infection taken up by sensory nerve terminals innervating the region of infection and carried by retrograde axonal transport to the neuronal cell body in the peripheral sensory dorsal root or trigeminal ganglion.⁴⁷ Recombinant nonreplicating HSV-based vectors are created by deletion of one or more essential IE genes. These recombinants retain the neuronal targeting properties of the wild-type virus and can be propagated to high titers on complementing cells but are unable to replicate in animals in vivo. Most reports have described the use of strong viral promoters (eg, the human cytomegalovirus immediate early promoter) to drive relatively high levels of transgene expression from the latent vector genomes. Other viral promoters, including the HSV ICP0 and ICP4 promoters, appear to function in a fashion similar to the HCMV IEp in driving transgene expression from the context of the vector genome. Prolonged transgene expression can be achieved using the HSV latency associated promoter (LAP2).⁴⁸

Replication-defective HSV vectors are nonpathogenic but retain the ability of wild-type virus to enter cells in the nervous system.⁴⁸ Because transduction is efficient and naturally targeted to neurons, relatively low titers of vector are required for therapeutic gene transfer. Overexpression of transgenes in primary afferents, neurons of spinal cord, or brain leading to the synthesis of releasable and antinociceptive molecules can be used to decrease the excitability of nociceptive neurons. Because production of antinociceptive proteins or derivative products from HSV vectors is continuous, this strategy is analogous to continuous local infusion. We have demonstrated the utility of these vectors in preclinical models of Parkinson disease, spinal root trauma, peripheral neuropathy, and pain.⁴⁸

Messages related to noxious thermal, mechanical, and chemical stimuli are transmitted from the skin and other peripheral sites to the CNS by myelinated A δ and unmyelinated C nociceptors. C fibers seem to release the excitatory amino acid and a variety of neuropeptides,

including substance P and calcitonin gene-related peptide.⁴⁹ Wilson et al⁵⁰ reported the first HSV-based vector expressing and releasing opiate peptides (enkephalin) in an acute pain model in the rat spinal cord. Hyperalgesia mediated by sensitization of C fibers or A δ fibers induced by laser stimulation after application of capsaicin or dimethyl sulfoxide, respectively, was reduced or eliminated in animals infected with the human proenkephalin coding HSV vector for at least 7 weeks postinfection.⁵⁰ This blockade of sensitization was reversed by intrathecal administration of the opioid antagonist naloxone, apparently acting in the spinal cord.⁵⁰ Antunes Bras et al⁵¹ found that 3 weeks after injection of a proenkephalin-encoding HSV vector into the footpad of rats, enkephalin RNA could be demonstrated in DRG by quantitative RT-PCR and in situ hybridization and that the peptide was carried by axonal transport to primary afferent terminals in spinal cord, resulting in a substantial increase in radioimmunoassayable met-enkephalin-like material in the dorsal spinal cord. Braz et al⁵² reported that rats with polyarthritis induced by an intradermal injection at the base of the tail of Freund adjuvant and injected with enkephalin-expressing HSV vector exhibited reduced thermal hyperalgesia and improved spontaneous locomotor activity; these effects were reversed by systemic naloxone or naloxone methiodide. We demonstrated that HSV-mediated delivery of preproenkephalin reduced spontaneous pain behavior during the second phase of the formalin test of inflammatory pain.⁵³ The naltrexone-reversible analgesic effect in inflammatory pain was maximal 1 week after vector inoculation, and could be reestablished by reinoculation of the vector after the initial effect has waned.⁵³ Yeomans et al⁵⁴ demonstrated that recombinant HSV vector encoding human preproenkephalin applied topically to the dorsal surface of the feet of the monkeys resulted in expression of enkephalin peptide in sensory neurons innervating the treated skin area. Behavioral responses evoked by capsaicin and activation of C nociceptors innervating the vector-treated skin area revealed a substantial and long-lasting (at least 20 wk) antihyperalgesic effect that was limited to areas to which the virus was applied.⁵⁴

Transgene delivery to brain neurons by replication-defective HSV vector was demonstrated by Kang et al⁵⁵ who reported that HSV-mediated delivery of preproenkephalin to the rat bilateral amygdala prevented most of the phase-2 flinching behavioral response in the formalin test. This study suggests that it may be possible to alter pain perception through gene transfer targeted at specific brain or brain stem nuclei.⁵⁵

In the spinal nerve ligation (SNL) model of neuropathic pain,⁵⁶ we reported that injection of an HSV vector expressing preproenkephalin 1 week after SNL produces a naloxone-reversible antiallodynic effect that is continuous and persistent for several weeks, and can also be reestablished by reinoculation of the vector after the original effect wanes.⁵⁷ In the neuropathic pain model, vector-mediated enkephalin expression enhances the effect of morphine, reducing the ED₅₀ of morphine from 1.8 to 0.15 mg/kg.⁵⁷ The vector continues to provide an antiallodynic effect in the face of tolerance to morphine induced by repeated injection of the drug.⁵⁷ Vector-mediated proenkephalin expression also blocks non-noxious touch-induced c-Fos expression in dorsal horn of animals with SNL.⁵⁷ We reported that HSV-mediated expression of proenkephalin reduces pain-related behavior in a rodent model of pain caused by cancer in bone.⁵⁸ In a rat model of trigeminal neuropathic pain which shares numerous similarities with human trigeminal neuralgia and trigeminal neuropathic pain,⁵⁹ Pohl and coworkers demonstrated that transgene-derived met-enkephalin significantly reduced mechanical hyperresponsiveness.⁶⁰ This antiallodynic effect was reversed by both the opioid receptor antagonist naloxone and the peripherally acting antagonist naloxone methiodide.⁶⁰

A substantial fraction of the primary nociceptors in DRG expresses the glial-derived neurotrophic factor (GDNF) receptor GFR α 1 and intrathecal administration of GDNF has been demonstrated to have an antinociceptive effect in the SNL model of neuropathic pain in rodents.⁶¹ We have reported that subcutaneous inoculation of an HSV vector designed to produce GDNF 1 week after SNL results in a significant reduction in mechanical allodynia that is

maximal 2.5 weeks after vector injection and subsequently declines.⁶² Like the studies with HSV-mediated expression of enkephalin, reinoculation with the GDNF-expressing vector after the effect of the initial inoculation waned reestablished the analgesic effect.⁶²

Among the complex mechanisms underlying neuropathic pain, partial nerve injury results in a selective loss of GABAergic inhibitory synaptic currents in spinal cord that contributes to abnormal pain sensitivity and the phenotypic features of the neuropathic pain syndrome.⁶³ We reported that subcutaneous inoculation of a replication-defective HSV vector expressing glutamic acid decarboxylase to increase synthesis of GABA after selective L5 SNL reversed mechanical allodynia and thermal hyperalgesia; the antiallodynic effect lasted 6 weeks and was reestablished by reinoculation and this vector also suppressed c-Fos immunoreactivity and pERK1/2 in the spinal cord dorsal horn.⁶⁴ We demonstrated that subcutaneous inoculation of this glutamic acid decarboxylase-expressing vector into both feet reduced mechanical allodynia and thermal hyperalgesia characteristic in spinal cord injury pain model.⁶⁵ The vector-mediated effect was partially reversed by intrathecal bicuculline or phaclofen indicating that the effect was mediated in part by both GABAA receptors and GABAB receptors.⁶⁵

Proinflammatory and anti-inflammatory cytokines and chemokines are endogenous small protein mediators released by local or migrating cells whose balance modulates the intensity of inflammatory response. The inflammatory stimuli or tissue injury induces the release of characteristic cytokine cascades, which ultimately triggers the release of final mediators responsible for inflammatory pain. IL-4 is one of anti-inflammatory cytokines, which functions to suppress IL-1 β , tumor necrosis factor- α (TNF α), and IL-8 production and to stimulate production of other anti-inflammatory peptides such as the IL-1 receptor antagonist.⁶⁶ IL-4 has been demonstrated to inhibit in a dose-dependent manner both acetic acid-induced writhing and zymo-san-induced inflammatory knee joint movement incapacitation.⁶⁷ We recently reported that subcutaneous inoculation of a nonreplicating HSV vector containing the IL-4 gene into the foot to transduce lumbar DRG and produce IL-4.⁶⁸ Transgene-mediated HSV vector expressing IL-4 did not alter thermal latency or tactile threshold in normal animals, but inoculation of the IL-4-expressing HSV vector 1 week after SNL reduced mechanical allodynia and reversed thermal hyperalgesia resulting from SNL.⁶⁸ Inoculation of this IL-4-expressing vector 1 week before SNL delayed the development of thermal hyperalgesia and tactile allodynia, although it did not prevent the ultimate development of these manifestations of neuropathic pain. Inoculation of the IL-4-expressing vector suppressed c-Fos immunoreactivity in dorsal horn of spinal cord and reversed the up-regulation of spinal IL-1 β , PGE2, and phosphorylated-p38 MAP kinase in the neuropathic pain.⁶⁸ TNF α soluble receptor, one of anti-inflammatory cytokines, can neutralize the effects of TNF α and revised allodynia induced by DRG compression.⁶⁹ We demonstrated that in the spinal cord injury model, TNF soluble receptor-expressing HSV vector inoculated into hindpaw reduced membrane-TNF α and phosphorylation of the p38 MAP kinase in the spinal dorsal horn, and painlike behavioral response.⁷⁰

Conclusions

In the last 10 years, substantial progress has been made in moving gene therapy for neurologic disease from a hypothetical possibility to a practical treatment. Current data indicates that several different vectors may be used to achieve focal gene transfer to central and peripheral nervous system to reduce pain-related behavior in different preclinical pain models. These animal data provides preclinical evidence to support the development of a human trial. A human trial of the proenkephalin-expressing vector in patients with intractable pain resulting from cancer metastatic to a vertebral body was reviewed by the Recombinant DNA Advisory Committee at the US National Institutes of Health (NIH) and is now under discussion with the

US Food and Drug Administration (FDA).⁷¹ Viral vector-based gene transfer may result in valuable novel therapies for the management of intractable chronic pain.

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