Published in final edited form as:
Neurosci Lett. 2012 October 11; 527(2): 85–89. doi:10.1016/j.neulet.2012.04.047.

Targeted Drug Delivery to the Peripheral Nervous System using Gene Therapy

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Abstract

Gene transfer to target delivery of neurotrophic factors to the primary sensory afferent for treatment of polyneuropathy, or of inhibitory neurotransmitters for relief of chronic pain, offers the possibility of a highly selective targeted release of bioactive molecules within the nervous system. Preclinical studies with non-replicating herpes simplex virus (HSV)-based vectors injected into the skin to transduce neurons in the dorsal root ganglion have demonstrated efficacy in reducing-pain related behaviors in animal models of inflammatory pain, neuropathic pain, and pain caused by cancer, and in preventing progression of sensory neuropathy caused by toxins, chemotherapeutic drugs or resulting from diabetes. Successful completion of the first phase 1 clinical trial of HSV-mediated gene transfer in patients with intractable pain from cancer has set the stage for further clinical trials of this approach.

Introduction

A drug is any substance used in the treatment, diagnosis, cure or prevention of disease. For many years, most drugs were small molecules either synthesized or isolated form plants, though beginning with insulin in the early 20th century and increasing rapidly in the era of molecular genetics, complex endogenous biochemical entities have been exploited to treat disease. An important pharmacologic principle that applies equally to endogenous biochemical entities as to small molecules is a dose-response relationship between the concentration of the drug and the magnitude of the desired effect. The upper bound of dose that may be administered to patients is generally restricted by toxicity, that in some cases results from the target pharmacologic action of the drug at the desired that is too great in magnitude, or may also occur as a pharmacologic effect on an undesired target. Off-target toxicity is particularly problematic in attempts to treat diseases of the peripheral nervous system (PNS), because of the wide anatomic distribution of most drugs' targets in the PNS,

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Disclosures

The Phase 1 and Phase 2 clinical trials were supported by Diamyd.

Dr. Wolfe is an employee of Diamyd and has an equity interest in the company. Dr. Wolfe and Dr. Fink are coinventors on patents that have been licensed by Diamyd. .

Dr. Mata and Dr. Fink have research supported by grants from the NIH and the Department of Veterans Affairs. Dr. Mata and Dr. Fink do not receive payments from Diamyd and do not any equity interest in Diamyd.

and the fact that many of the desired molecular targets in the PNS are also found in the central nervous system and in non-neural structures.

Gene therapy, while initially proposed as a strategy that could be used to correct inherited genetic defects [11], can also be used to deliver short-lived bioactive molecules to restricted anatomic locations. This approach has been exploited in gene therapy approaches to focal CNS diseases such as Parkinson's Disease [21], where a wide range of viral vectors including adenoassociated virus-based vectors and lentiviral vectors have be used to express neurotrophic factors or enzymes in specific brain regions. The peripheral nervous system however poses some special challenges. Sensory neurons, with their cell bodies located in the dorsal root ganglia, are widely distributed and relatively inaccessible to direct injection, and the pseudo-unipolar axons projecting peripherally to the target organ and centrally to the spinal cord are very large in comparison to the size of the cell body.

Among the several gene transfer vectors that are available, HSV is particularly well suited for gene delivery to the PNS. HSV possesses a natural tropism for peripheral sensory neurons of the dorsal root ganglion (DRG), where the virus naturally establishes a latent state in which viral genomes persist for the life of the host as intranuclear episomal elements. The life-long persistence of latent genomes in trigeminal ganglion without the development of sensory loss or histologic damage to the ganglion attests to the effectiveness of these natural latency mechanisms. Wild-type virus may be reactivated from latency under the influence of a variety of stresses, but recombinant vectors that are entirely replication defective retain the ability to establish persistent quiescent genomes in neurons, but are unable to replicate (or reactivate) in normal cells, including those of the nervous system.

Over the past decade, we have made substantial progress in developing HSV vectors for gene transfer to sensory neurons, and in 2010 completed the first phase 1 clinical trial of HSV-mediated gene transfer in patients. There are two categories of disease processes that we judge are particularly appropriate for targeted delivery using HSV vectors: sensory polyneuropathy and chronic pain. Polyneuropathy refers broadly to a family of conditions in which a peripheral sensory axons degenerate, often in a length-dependent fashion. With the exception of immune mediated neuropathies that can be treated by immunomodulation, there are no currently available treatments to effectively treat or prevent the progression of neuropathy resulting from systemic illness (e.g. that caused by diabetes), toxic exposure (e.g. chemotherapy induced peripheral neuropathy) or genetic defect (e.g. Charcot-Marie-Tooth disease).

Extensive preclinical animal studies beginning in the 1990s demonstrated that neurotrophic factors delivered by intra-peritoneal injection could effectively prevent the progression of polyneuropathy resulting from any one of a number of causes including diabetes, toxic exposures or genetic defect. Subsequent work extended the range of factors from the classical neurotrophins (e.g. nerve growth factor, neurotrophin-3) to other peptides with neurotrophic effects including insulin like growth factor, vascular endothelial growth factor, and erythropoietin. However, despite the abundant evidence that systemic administration of these factors is effective in preventing the progression of neuropathy in animal models, clinical trials failed to demonstrate a therapeutic effect in patients. While there are many possible explanations for these discordant results, one obvious problem is that the dose of peptide factor utilized in the animal studies - typically in the range of 5 to 10 mg/kg - was much higher than the doses tolerated by human subject. In the phase 3 clinical trial of NGF for diabetic neuropathy a dose of 0.1 μg/kg proved ineffective in preventing the progression of neuropathy in these patients.

Pain is a complex experience with sensory discriminative, cognitive and emotional components. Acute pain is initiated by activation of a subset of sensory afferents (nociceptors), transmitting nociceptive information centrally through a well-characterized ascending pathway that serves to warn the individual of potentially harmful stimuli in the environment often leading to a reflex withdrawal response. Chronic pain is the percept that is produced in the setting of tissue damage (often called inflammatory or nociceptive pain) or after damage to the neural structures serving pain perception. Chronic pain has many of the same unpleasant affective and cognitive aspects as acute pain, but characteristically leads to reduced activity and avoidance of contact; phenomena we measure experimentally as allodynia and hyperalgesia. Although the dorsal root ganglion is neither necessary for nor sufficient for the experience of chronic pain, most of the common forms of chronic pain proceed through the same anatomic pathways as those utilized for acute pain, a pathway that involves a first order synapse in the dorsal horn of the spinal cord.

Nociceptive neurotransmission at the first synapse in the dorsal horn between the primary nociceptor of the PNS and second-order CNS neurons projecting rostrally is subject to complex modulatory influence mediated by excitatory and/or inhibitory neurotransmitters released from interneurons under the control of descending inputs. Pharmacologic activation of inhibitory neurotransmitter (predominantly but not limited to opioid and GABA) receptors presynaptically on primary afferents or postsynaptic and second-order neurons represents one effective means of down-modulating chronic pain. However, the same receptors are widely distributed throughout the central neuraxis and in the case of opioids on non-neural structures as well so that off target effects unrelated to analgesia that are elicited by systemic administration of opiates or GABA-active drugs limit be used for pain relief. Intrathecal administration to target drugs to spinal targets for example, allows one to increase the effective dose tenfold.

Gene transfer to target delivery of neurotrophic factors to the primary sensory afferent for treatment of polyneuropathy, or of inhibitory neurotransmitters for relief of chronic pain, offers the possibility of a highly selective targeted release of bioactive molecules within the nervous system. Release of neurotrophic factors from transduced primary sensory afferents could, through autocrine and paracrine effects, protect sensory neurons from degeneration without requiring high-dose systemic delivery. Release of inhibitory neurotransmitters from primary sensory afferents terminals in the dorsal horn could provide an analgesic effect without the side effects engendered by activation of these receptors in other sites nervous system or other tissues.

Biology of HSV

The HSV particle consists of a nucleocapsid surrounded by an envelope containing glycoproteins essential for virus attachment and penetration into cells. The HSV genome contains 152 kb of linear, double-stranded DNA expressing approximately 90 unique transcriptional units organized into two segments, a unique long (UL) and unique short (US) segment, each of which is flanked by inverted repeats containing important IE and latency genes. The viral genes are almost entirely found as contiguous transcribable units, making their genetic manipulation relatively straightforward. In wild type infection, the virus is transmitted by direct contact, replicating initially in epithelial cells of skin or mucous membranes. Second generation virions are taken up by sensory nerve terminals and carried by retrograde axonal transport to the neuronal perikaryon in DRG where viral DNA is injected through a modified capsid penton into the nucleus. In the lytic replication cycle expression of the viral IE genes (which occurs in the absence of *de novo* protein synthesis) serves to transactivate expression of early (E) genes. Removal of essential IE genes from the HSV genome results in vectors which are unable to enter the lytic cycle in non-

complementing cells, but nonetheless are transported in a normal fashion to the nucleus where they establish a persistent latent state [10, 20, 27]. The latent state occurs naturally only in neurons. In this state, following injection of the viral genome into the nucleus, expression of the gene products characteristic of lytic infection is repressed and the viral genome persists as an intranuclear circularized episomal element. Latent genomes continue to transcribe only one segment of the inverted repeat sequences in the inverted repeat flanking UL, just downstream of and from the strand opposite the IE ICP0 gene to produce a family of latency-associated transcripts (LATs). Latent genomes are partially methylated and sequestered as an episomal minichromosome-like structure bound by nucleosomes, and have no discernible effect on host cell metabolism or phenotype. Non-replicating vectors constructed by deletion of essential IE genes are therefore forced into a pseudolatent state.

Preclinical studies of HSV gene transfer for pain

The efficacy of HSV-mediated gene transfer of enkephalin has been tested in several different models of pain in rodents. Pohl and co-workers first showed that a replication competent, *tk*-defective HSV recombinant injected subcutaneously in the paw will transduce DRG neurons and express enkephalin in DRG [2]. Wilson and co-workers subsequently demonstrated that a similar tk^- herpes simplex virus (HSV) based vector containing the human proenkephalin gene and injected subcutaneously in to the paw, reduces hyperalgesic C-fiber responses ipsilateral to the injection [26]. Pohl and co-workers went on to show that subcutaneous inoculation of the vector reduces pain-related behaviors in a rodent model of chronic pain related to polyarthritis induced by injection of complete Freund's adjuvant (CFA) [3]. Expression of enkephalin from the vector not only reduced pain-related behaviors, but also prevented cartilage and bone destruction in the inflamed joints, presumably because of release of enkephalin, which has both anti-nociceptive and anti-inflammatory effects, from the peripheral sensory terminals in the joint [3], an observation that was supported by the observation that the transgene product was carried by axonal transport both towards the periphery as well as towards the spinal cord [1].

Subcutaneous inoculation of an enkephalin-producing replication defective, essential IE gene deleted vector produces an analgesic effect in the delayed phase of the formalin model of inflammatory pain [15] in the selective spinal nerve ligation model of neuropathic pain [16], and in the infraorbital nerve constriction model of craniofacial pain [24].

In experiments designed to test the effect of the vector on visceral pain, investigators have injected the vector directly into the end organ, rather than the skin. Yoshimura *et al.* demonstrated that injection of the replication defective enkephalin-expressing HSV vector into the rat bladder wall results in enkephalin expression in relevant DRG, and that vector-mediated enkephalin effectively attenuated capsaicin-induced bladder irritation and resultant bladder hyperactivity [12, 33]. Similarly, Westlund and colleagues have shown in rodent models of acute and chronic pancreatitis that direct injection of an enkephalin-expressing HSV vector into the pancreas attenuates evoked nocisponsive behaviors [23, 31]. In the pancreas, enkephalin expression appeared to reduce the inflammatory response in the pancreas, analogous to the effect that reported in polyarthritis [3]. In a mouse model of bone cancer pain, subcutaneous inoculation of the replication defective HSV vector expressing enkephalin resulted in an attenuation of spontaneous nocisponsive behaviors [14].

Studies of the enkephalin-expressing HSV vector have been extended to primates. Yeomans *et al.* demonstrated that peripheral application of a replication competent HSV vector expressing enkephalin to the dorsal surface of the foot of macaques reduced a A-delta and C-fiber mediated pain-related responses [32]. Taken together, these results from several different groups of investigators provide proof-of-principle evidence that HSV vector-

mediated delivery of enkephalin can provide an analgesic effect, and set the stage for a human trial to treat chronic pain using HSV vector-expressed enkephalin.

Our groups have examined HSV vectors constructed to express other inhibitory neurotransmitters. Endomorphin-2 (EM-2; Tyr-Pro-Phe-Phe-NH2) is an endogenous highly selective mu receptor agonist [34], but the gene coding for EM-2 has not yet been identified. We therefore constructed a tripartite synthetic gene cassette with the N terminal signal sequence of human preproenkephalin followed by a pair of endomorphin-2 coding elements including the addition a C-terminal glycine residue flanked by dibasic cleavage sites. The gene product is processed by the cellular machinery that processes preproenkephalin to enkephalin [28], with the C terminal glycine in the cleaved product directs amidation of the cleaved peptide by the widely distributed enzyme peptidylglycine α-amidating monooxygenase. Subcutaneous inoculation of the replication defective endomorphin expressing HSV vector into the footpad of rats with neuropathic pain from selective L5 spinal nerve ligation resulted in a significant reduction in both mechanical allodynia and thermal hyperalgesia that could be blocked by the highly selective μ-opioid receptor antagonist CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr amide) [28], and a substantial reduction in nocisponsive behaviors in the delayed phase of the formalin test and in the complete Freund's adjuvant model of inflammatory pain [18].

Nocisponsive behaviors can also be attenuated by using HSV-mediated gene transfer to modulate expression of mu opioid receptors in primary sensory afferents. Cutaneous application of a replication competent, *tk*-defective HSV vector with the human mu opioid receptor cDNA in reverse orientation results in decreased expression of mu opioid receptors on the central primary sensory afferent terminals in the dorsal horn of spinal cord resulted in a reduced potency of intrathecal [D-Ala^2,*N*-MePhe^4,Gly-ol^5] enkephalin (DAMGO) on C-fiber nociceptive responses [19] consistent with a centrally mediated effect. Conversely, cutaneous application of an HSV vector expressing the mu opioid receptor gene in the sense orientation increases mu opioid receptor immunoreactivity in primary sensory afferents and a leftward shift in the dose-response to intraperitoneal lopiramide, indicating an effect at transgene-mediated mu opioid receptors expressed on the peripheral terminals of the primary sensory neurons [35].

We constructed a replication defective HSV vector encoding the 67 kD isoform of human glutamic acid decarboxylase (GAD67) [22]. In the selective spinal nerve ligation model of neuropathic pain inoculation of the GAD-expressing vector resulted in a substantial reduction in mechanical allodynia and thermal hyperalgesia [17]. In neuropathic pain the analgesic effect of the GAD-expressing vector is greater in magnitude than the effect produced by either the enkephalin or endomorphin-expressing vectors. This finding is consistent with the evidence that development of chronic pain after peripheral nerve injury is accompanied by the loss of GABAergic tone in the dorsal horn of spinal cord [25], and the clinical observation that opiate drugs are relatively ineffective in the treatment of pure neuropathic pain. The GAD-expressing HSV vector also reduces pain-related behaviors in a model of central neuropathic pain created by T13 spinal cord hemisection [22].

Preclinical studies of HSV-mediated gene transfer in models of polyneuropathy

Over the past decade, we have completed extensive preclinical studies of HSV-mediated gene transfer in several different models of neuropathy. In the model of selective large myelinated fiber degeneration caused by high-dose pyridoxine (PDX), subcutaneous inoculation of a non-replicating HSV vector coding for NT-3 resulted in preservation of sensory nerve amplitude, sensory nerve conduction velocity, and amplitude of the H-wave;

protection of large myelinated fiber proprioceptive sensory function, and preservation of large myelinated fibers in nerve and in the dorsal horn of spinal cord [8]. In a model of toxin-induced neuropathy caused by cisplatin, subcutaneous inoculation of HSV vectors constructed to express either NGF or NT-3 just prior to a 6-week course of cisplatin resulted in significant protection against the development of neuropathy assessed by electrophysiologic, behavioral and morphologic outcomes [4]

In the model of type 1 diabetes in Swiss Webster mice created by injection with streptozotocin (STZ) subcutaneous inoculation of a replication defective HSV vector expressing either nerve growth factor [13], vascular endothelial growth factor [5] or erythropoietin [7] into both hind feet 2 weeks after the induction of diabetes prevented the loss of sensory nerve action potential amplitude characteristic of neuropathy measured 4 and 8 weeks after the injection of STZ.

In these initial studies we employed the human cytomegalovirus immediate early promoter (HCMV IEp) to drive transgene expression, and examined the biological effect of vector-mediated transgene expression up to 2 months after inoculation. To achieve prolonged transgene expression we employed the HSV latency-associated promoter 2 (LAP2) element (nucleotides 118866 – 119461 of the HSV genome). LAP2 is the sequence responsible for lifelong expression of latency associated transcripts in neurons infected with wild-type virus. Using a vector with the LAP2 promoter driving expression of neurotrophin-3 (NT-3) we found that five and a half months after vector inoculation, animals inoculated with the LAP2-driven NT-3-expressing replication defective vector showed preservation of peripheral nerve function in the face of subacute PDX intoxication [9]. Similarly, in the STZ diabtes model, mice inoculated with the NT-3 expressing were protected against the progression of diabetic neuropathy over the course of 6 months [6].

Because prolonged expression of neurotrophic factors could potentially have unwanted adverse effects, we constructed a replication defective HSV vector vHrtEPO, to express erythropoietin (EPO) under the control of a tetracycline response element (TRE)-minimal CMV fusion promoter. Primary DRG neurons in culture infected with vHrtEPO express and release EPO in response to exposure to doxycyline (DOX). Animals infected with vHrtEPO by footpad inoculation demonstrated regulated expression of EPO in DRG under the control of DOX administered by gavage. Mice rendered diabetic by injection of streptozotocin, inoculated with vHrtEPO and treated with DOX 4 days out of 7 each week for 4 weeks were protected against the development of diabetic neuropathy assessed by electrophysiologic and behavioral measures [29]. These studies indicated that intermittent expression of EPO in DRG achieved from a regulatable vector is sufficient to protect against the progression of neuropathy in diabetic animals, and provides proof-of-principle preclinical evidence for the development of such vectors for clinical trial.

In a subsequent study, we constructed an HSV vector with expression of EPO under the control of the Tet-on system in which the HSV latency-associated promoter 2 (LAP2) element was used to drive the expression of the Tet-on transactivator. Mice with STZ-induced diabetes inoculated with the LAP2 regulatable EPO-expresing vector were protected against the development of neuropathy when given continuous administration of DOX-containing chow over the course of 3 months [30]. Identical results were achieved when DOX was administered every other week over 3 months of diabetes, but administration of DOX 1 week out of 3 provided only partial protection against the development of neuropathy. Taken together, these results suggest such a vector is well suited for clinical trial for the treatment of chronic or subacutely developing neuropathy.

Clinical trials of the preproenkephalin-expressing HSV vector

Based on the preclinical data in the cancer pain model, and supported by FDA-approved toxicology and biodistribution studies, we conducted a multicenter, dose-escalation, Phase I clinical trial of NP2, a replication defective HSV-based vector expressing human preproenkephalin (PENK) in subjects with intractable focal pain caused by cancer. NP2 was injected intradermally into the dermatome(s) corresponding to the radicular distribution of pain. The primary outcome was safety. As secondary measures, efficacy of pain relief was assessed using a numeric rating scale (NRS), the Short Form McGill Pain Questionnaire (SF-MPQ) and concurrent opiate usage.

Ten subjects with moderate to severe intractable pain despite treatment with more than 200 mg/day of morphine (or equivalent) were enrolled into the study. Treatment was well tolerated with no study agent-related serious adverse events (SAE) observed at any point in the study. Subjects receiving the low dose of NP2 reported no substantive change in pain. Subjects in the middle and high dose cohorts reported pain relief as assessed by NRS and SF-MPQ. There were no placebo controls in this relatively small study, but the dose-responsive analgesic effects were encouraging, and a Phase 2 placebo-controlled trial is underway.

Conclusion

Conditions of the PNS represent a challenge for the development of novel therapies. The preclinical studies described in this review provide strong support from animal models to suggest that intractable focal pain unresponsive to conventional treatment, and chronic sensory polyneuropathy, a condition for which there is currently no treatment, may both be amenable to targeted drug delivery by gene transfer to the DRG using HSV-based vectors. The results of the Phase 1 trial, that represents both the first test of an HSV-based vector for gene transfer in patients and the first gene therapy trial for pain, is an important step towards moving this form for treatment from the laboratory into the clinic. We are moving forward towards testing of a GAD-expressing vector in treatment of neuropathic pain and a neurotrophin-expressing vector in patients at risk for chemotherapy-induced neuropathy in patients. The results of the Phase 2 trial of encephalin and those two additional studies will be crucial in determining whether this approach can be brought into the clinic in the next few years.

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Highlights

- HSV-based vectors are particularly suitable for delivering genes to the DRG
- Preclinical studies in pain and for treatment of polyneuropathy are reviewed
- Results of the first phase 1 clinical trial support this approach