A Clinical Trial of Gene Therapy for Chronic Pain

Darren Wolfe, PhD,* James Wechuck, PhD,* David Krisky, MD, PhD,* Marina Mata, MD,[†] and David J. Fink, MD[†]

*Diamyd Inc., Pittsburgh, Pennsylvania; [†]University of Michigan and Ann Arbor VA Healthcare System, Ann Arbor, Michigan, USA

ABSTRACT

The first human trial of gene therapy for chronic pain, a phase 1 study of a nonreplicating herpes simplex virus (HSV)-based vector engineered to express preproenkephalin in patients with intractable pain from cancer, began enrolling subjects in December 2008. In this article, we describe the rationale underlying this potential approach to treatment of pain, the preclinical animal data in support of this approach, the design of the study, and studies with additional HSV-based vectors that may be used to develop treatment for other types of pain.

Key Words. Pain Management; Persistent Pain; Neurology; Therapy

Introduction

A critical limitation in the use of standard oral drugs to treat severe chronic pain is that their actions are not limited to the neural pathways involved in the transmission of pain-related information [1]. As a result, effects of these drugs on neural pathways unrelated to pain or on organs outside the nervous system pose substantial limitations on the maximum dose that may be administered. Opiate drugs such as morphine provide an excellent example of this phenomenon. These drugs exert their analgesic effect through binding to endogenous opiate receptors in the nociceptive pathway [2]. But the same receptors are present throughout the nervous system resulting in lethargy, confusion, and respiratory suppression. At lower doses, binding of these drugs to opiate receptors in the gut produces constipation and activation of receptors in the urinary tract results in urinary retention. The use of standard opiate drugs can be further complicated by the development of tolerance and the broad potential for addiction and abuse.

In order to exploit the analgesic properties of endogenous inhibitory neurotransmitters on nociceptive neurotransmission while avoiding the off-target effects of these potent bioactive peptides on other neural and non-neural pathways, we and others have been exploring the use of gene transfer to provide continuous focal production and release of inhibitory neurotransmitters directly in the affected peripheral nervous system. In previous studies, we have focused on the first synapse between the primary nociceptors and the second-order nociceptive projection neuron in the dorsal horn of the spinal cord by transferring the required genes into the primary sensory afferent in the dorsal root ganglion (DRG) using nonreplicating gene transfer vectors created from herpes simplex virus type 1 (HSV-1) [3].

The first human trial of gene therapy for chronic pain, a phase 1 study of a nonreplicating HSV-based vector engineered to express preproenkephalin in patients with intractable pain from cancer, began enrolling subjects in December 2008. In this article, we describe the preclinical animal data in support of this approach, the design of the study, and studies with additional HSV-based vectors that may be used in the development of treatment for other types of pain.

HSV-Mediated Gene Transfer to Primary Sensory Neurons

HSV is an enveloped, double-stranded DNA virus that, in its wild-type form, causes cold sores. We chose HSV to create the gene transfer vectors for

Reprint requests to: David J. Fink, MD, University of Michigan and Ann Arbor VA Healthcare System, 1914 Taubman Center, Ann Arbor MI 48109. Tel: 734-936-9070; Fax: 734-763-5059; E-mail: djfink@umich.edu.

this purpose because HSV is a naturally neurotropic virus that is spread by contact with the skin, after which, it is efficiently taken up by nerve terminals and transported along axons to establish a persistent (latent) state in sensory neurons of the DRG or trigeminal ganglion [4]. Nonreplicating (inert) gene transfer vectors were constructed from wild-type HSV by selective deletion of essential immediate early genes from the HSV genome [5]. These replication-incompetent gene transfer vectors can be propagated to high titer in complementing cells that provide the missing essential immediate-early gene products from within the complementing cell genome [6]. Injected into animals (or people), the vector is incapable of replication but retains the other biological properties of the parental virus so that the vector is taken up from the skin by sensory nerve terminals and carried to the DRG where the genome establishes a persistent state as a nonintegrated (episomal) intranuclear element [4].

Preclinical Studies of the Preproenkephalin-Expressing HSV Vector

The most extensively studied of the HSV-based gene transfer vectors for pain has been engineered to contain the gene coding for human preproenkephalin, a precursor peptide that contains six copies of met-enkephalin and one copy of leu-enkephalin [7]. Transduction of primary DRG neurons in tissue culture with this vector results in the release of substantial amounts of enkephalin into the medium, indicating that the gene product is packaged into vesicles and processed properly by the endogenous cellular machinery. Injection of the vector subcutaneously into the plantar surface of the hind paw of rats results in transduction of neurons in the ipsilateral lumbar DRG, as determined by polymerase chain reaction (PCR), reverse transcriptase (RT)-PCR, and immunocytochemistry [7,8]. The vector provides a significant analgesic effect in animals with arthritis induced by injection of complete Freund's adjuvant [9], in the delayed phase of the formalin test (pain induced by injection of formalin into the paw) [7], in facial pain created by constriction of the infraorbital nerve [10], in neuropathic pain caused by selective ligation of the L5 spinal nerve [11], and in pain caused by cancer in bone [7].

In these studies, we and others have shown that the effect of the vector-mediated enkephalin can be reversed by administration of opiate receptor antagonists (naloxone and naltrexone). The analgesic effect of the vector-mediated enkephalin expression is continuous throughout the day and persists for several weeks [11], consistent with the time course of the human cytomegalovirus immediate-early promoter used in this vector to drive preproenkephalin expression. The effect can be reestablished by reinoculation of the vector after the analgesic effect of the original injection has waned. The analgesic effect of the vector is additive with the effect of systemically administered morphine, shifting the dose–response to morphine in animals with neuropathic pain from selective L5 spinal nerve ligation (SNL) from 1.8 mg/kg in animals inoculated with vehicle or control vector to 0.15 mg/kg in animals inoculated with the enkephalin-expressing vector. In addition, we found that vector-mediated analgesic effect persisted in animals rendered tolerant to the analgesic effects of morphine [11].

Interestingly, we found that activation of the delta opioid receptor by continuous release of enkephalin from transduced neurons does not only block pain-related behaviors but appears to alter the phenotype of DRG neurons [12]. In a model of painful diabetic neuropathy (PDN), for example, the NaV1.7 tetrodotoxin-sensitive voltage-gated sodium channel isoform plays a critical role in nociception and has been found to be increased in DRG neurons in correlation with the emergence of pain-related behaviors characteristic of PDN. Transduction of DRG neurons by subcutaneous inoculation of the HSV-based vector expressing preproenkephalin reversed nocisponsive behavioral responses to heat, cold, and mechanical pressure characteristic of PDN, and prevented the increased expression of NaV1.7 in the DRG characteristic of PDN, an effect that correlated with inhibition of phosphorylation of p38 mitogen-activated protein (MAP) kinase and protein kinase C (PKC). The same effects were observed in primary DRG neurons in vitro exposed to 45 mM glucose for 18 hours and treated with the vector. The in vitro effect of the vector on NaV1.7 levels was prevented by naltrindole [12]. The results of these studies suggest that activation of the presynaptic delta opioid receptor by enkephalin prevents the increase in neuronal NaV1.7 in DRG through inhibition of PKC and p38.

Taken together, the results summarized above demonstrate that an HSV vector expressing preproenkephalin effectively reduces pain in rodent models of chronic pain due to inflammation, nerve

injury, and pain resulting from cancer in bone. The relative effectiveness of expression of this opiate peptide suggests that it might be particularly useful in the treatment of inflammatory or cancer pain.

Preclinical Studies of Gene Transfer of Glutamic Acid Decarboxylase (GAD) to Treat Neuropathic Pain

Neuropathic pain is particularly resistant to treatment with available agents. Because there is substantial evidence implicating reduced spinal GABAergic inhibition in neuropathic pain [13,14], we constructed a vector expressing GAD to produce gamma amino butyric acid (GABA) in transduced cells. Expression and release of GABA from cells transduced by the GAD-expressing vector was confirmed by microdialysis in primary DRG neurons in vitro and in dorsal horn of spinal cord in vivo after subcutaneous inoculation of the vector in the foot of rats [15]. GABA produced by transgene-mediated GAD67 is likely located in the cytoplasm, rather than in vesicles, and release of GABA from transduced neurons appears to occur through reversal of the GABA transporter (GAT-1) as indicated by the observation that release from transduced neurons was inhibited by the GAT-1 inhibitor N0-711 in a dose-dependent manner [16].

In the T9 hemisection model of central neuropathic pain resulting from spinal cord injury, subcutaneous inoculation of the GAD-expressing vector in the feet resulted in a substantial reduction in mechanical allodynia and thermal hyperalgesia in the hind limbs [15]. The antinociceptive effect of GABA released from the vector-transduced cells was blocked by intrathecal injection of bicuculline or phaclofen [15] indicating that the vector-produced effect was mediated in part by both GABAA and GABAB receptors. In the selective SNL model of neuropathic pain [17], subcutaneous inoculation of the GAD-expressing vector 1 week after SNL produced a substantial antiallodynic effect that peaked about 2 weeks after inoculation and persisted for 6 weeks [18]. The antinociceptive effect of the GAD vector was greater than that of vector-mediated enkephalin or endomorphin in this model. Like the antiallodynic effect of the enkephalin-expressing vector, the antiallodynic effect of GAD vector inoculation waned over a time course of weeks, but was reestablished by reinoculation with the same vector.

At 6 weeks after injection of streptozotocin, diabetic rats demonstrated: thermal hyperalgesia manifested by a decrease in withdrawal latency in response to noxious thermal stimuli compared with the control animals; cold allodynia manifested by a decrease in response latency to acetone; and mechanical hyperalgesia measured using the Randall Sellito method. Diabetic animals inoculated with the GAD vector showed a significant reversal of each of these nocisponsive parameters. Four weeks after inoculation (6 weeks after induction of diabetes), we observed a statistically significant increase in thermal latency, a statistically significant reversal of cold allodynia, and a significant reversal of mechanical hyperalgesia in GAD vector-inoculated diabetic animals. GAD protein was detected by immunocytochemistry in DRG neurons in vivo 1 week after inoculation.

Phase 1 Human Trial of HSV-Mediated Gene Transfer for Pain

On the basis of the encouraging results from preclinical studies of the preproenkephalin-expressing vector in the several different models of chronic pain in animals, we were interested to proceed to test this approach in patients with chronic pain. Because HSV-mediated gene transfer had not previously been tested in humans, we chose to perform the first phase 1 safety/dose finding study in patients with intractable pain from terminal cancer. To produce a high-titer vector stock while eliminating the potential of homologous recombination producing replication-competent recombinants, the replication defective HSV backbone was reengineered to completely remove homology between the complementing cell line and the vector genomes [19]. As an additional safety feature, the human-grade vector was engineered so that the transgene (preproenkephalin) was inserted in place of both copies of the essential immediate-early HSV gene *ICP4*; were recombination with wild-type virus to occur, recombination of the preproenkephalin gene into the wild-type genome would render the resulting genome unable to replicate.

Extensive toxicology and biodistribution studies of the clinical trial-grade enkephalin-expressing vector were carried out according to Food and Drug Administration (FDA) guidance. Ten mice/sex/group/time point were injected with three different doses of the vector and sacrificed

on days 1, 7, 28, and 91 postdosing. There was no evidence of adverse treatment-related effects of the test article as judged by clinical observations, body weight, or food consumption. Clinical pathology revealed no significant changes in hematology or clinical chemistry parameters at each time point. There were no treatment-related abnormalities in organ weight and no gross macroscopic and no microscopic abnormalities were associated with vector treatment. Based on the predefined parameters of the toxicology study, administration of the test article according to the conditions of this study was well tolerated and was without significant toxicity. A panel of tissues harvested from the in-life phase of a parallel biodistribution study were examined for vector biodistribution. Quantifiable vector sequences were found only in the injection site, underlying muscle, and associated DRG; there was no quantifiable dissemination to any other tissue. On the basis of preclinical efficacy, toxicological and biodistribution safety data, and vector quality testing, we received FDA investigational new drug (IND) approval for the human trial.

This study will enroll 12–24 patients with intractable focal pain from cancer. In order to be considered for the study, patients must have terminal cancer (less than 12 months projected survival in the estimation of the treating oncologist) and pain that is greater than 4 out of 10 on a visual analog scale despite treatment with greater than 200 mg/day of morphine (or equivalent). Patients with unstable medical disease, chemotherapy within the past 3 weeks, or radiation within the past 14 days are excluded. During the clinical phase, patients are allowed to continue with their usual treatment regimen and pain medications as prescribed and adjusted by their treating physician.

Eligible patients will receive 10 intradermal injections of 100 uL each into the dermatome corresponding to the radicular distribution of their pain. As a phase 1 trial, the primary outcome is to determine the safety of vector injection. This will be assessed by physical examinations as well as hematologic and virological analyses performed on day 3, 7, 10, 14, 21, 28 and monthly thereafter. Pain will be assessed as a secondary outcome using a numeric rating scale [20], the McGill Short-Form Pain Questionnaire [20], the Eastern Cooperative Oncology Group (ECOG) Performance Status [21], and concurrent analgesic use assessed by patient diary. In this dose-escalation trial, three patients will receive 10^7 plaque-forming units (pfu)

of the vector, three patients will receive 10^8 pfu, and three patients will receive 10^9 pfu. At the highest tolerated dose, an additional three patients will be enrolled. The trial can be expanded to include 12 additional patients at the highest dose to further investigate safety and/or efficacy if desired.

Additional Approaches to Treating Chronic Pain Using HSV-Based Vectors

The phase 1 clinical trial currently underway will establish the safety of the HSV-mediated approach in patients. The preclinical animal data indicate that while preproenkephalin gene transfer should be useful for the treatment of inflammatory pain, it is not the most effective vector for the treatment of neuropathic pain. Therefore, based on the preclinical data, we have submitted a proposal for a phase 1/2a trial of the GAD-expressing vector in patients with neuropathic pain from PDN. This proposal was reviewed by the Recombinant DNA Advisory Committee of the National Institutes of Health (NIH) in March 2009. In addition, we have constructed an HSV-based vector to express endomorphin-2 [22], one of the putative endogenous ligands for the mu opioid receptor, that will follow. Beyond these applications at hand, HSV vectors expressing a number of other gene products of interest in the treatment of pain are being studied in animal models. Yeomans and colleagues have shown that HSV vectors expressing antisense RNA sequences can be used to reduce expression of voltage-gated sodium channels [23] or the pain-related neurotransmitter calcitonin gene-related peptide (CGRP) [23] in primary sensory afferents with concurrent pain-relieving results. In light of the important potential role of a neuroimmune response in the pathogenesis of neuropathic pain, we have constructed and tested HSV vectors expressing the anti-inflammatory peptides IL-4 [24], IL-10 [25], and the truncated form of the p55 TNFá receptor [26–28], all of which reduce the spinal inflammatory response concurrent with a reduction in pain in rodent models. And in a novel approach, HSV-mediated expression of dominant negative form of PKC has been used to reduce basal and sensitizing responses to capsaicin in peripheral nerve [29].

Conclusion

Extensive preclinical studies published over the past decade have shown that HSV-based vectors

injected into the skin can be used to transduce peripheral sensory neurons to reduce pain-related behaviors in a variety of rodent models of chronic pain. These studies have moved from the laboratory to patients, with the first human trial of gene therapy for chronic pain using an HSV-based vector expressing preproenkephalin in patients with terminal cancer pain. A second trial, to examine the effect of an HSV vector expressing GAD in neuropathic pain is in the wings. The results of these studies will determine whether gene transfer will prove useful in the treatment of patients with chronic focal forms of inflammatory or neuropathic pain.

Financial Disclosure Statement

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