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Applications of Gene Therapy to the Treatment of Chronic Pain

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Abstract

Chronic pain is a highly prevalent condition that impacts adversely on individual quality of life, imposes substantial costs on the healthcare system and a considerable burden on society. Advances in the understanding of pain mechanisms have opened the way for the development of new treatment strategies. The continuous delivery of short-lived potent bioactive molecules to sensory nerves, spinal cord or meninges - achieved by directed gene transfer - offers the possibility to selectively interrupt nociceptive neurotransmission or to interfere with the plastic changes in the nervous system underlying the development or persistence of chronic pain. In this review we describe advances in the use of non-viral and viral vector-based gene transfer for the treatment of pain, with a special focus on the use of recombinant non-replicating herpes simplex virus-based vectors and the prospects for clinical trials.

Keywords

pain; enkephalin; endomorphin; gamma aminobutyric acid; cytokines; trophic factors; RNA interference; herpes simplex virus

Introduction

Pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” [1]. Acute pain perception is adaptive, and critical for healthy survival. In a number of settings, though, the persistence of pain long after the inciting injury has resolved, or persistent pain resulting from primary nerve or tissue damage that cannot be resolved is maladaptive. These syndromes, referred to collectively as “chronic pain”, represent a major cause of morbidity, significantly impairing individual quality of life [2] and imposing a substantial societal burden. The neuroanatomic pathways and neurotransmitter systems responsible for acute pain perception are similarly involved in the perception of chronic pain, but the experience of chronic pain is not the simple result of continuous activation of nociceptive pathways. Alterations in transcription and protein translation in neurons and in glia, changes in electrophysiologic activity of neurons, and anatomic reorganization all may occur at various levels in the pain pathway, ranging from the peripheral nociceptors in the dorsal root ganglion (DRG) to third-order neurons in the thalamus and in projections to sensory and limbic cortex in chronic pain.

In the simplest case, chronic pain can be relieved by a treatment that removes or eliminates the source of the injury. A good example of this phenomenon is the resolution of chronic hip pain following replacement of the damaged joint by a prosthetic joint. However, in many cases the pain-inciting tissue damage cannot be simply removed, necessitating the use of a

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progressive increasing drug treatment “ladder” that begins with non-steroidal anti-inflammatory drugs to reduce peripheral inflammation and coincidentally block neuro-inflammatory changes in the dorsal horn of spinal cord, progressing through the use of mild opioid drugs (e.g. codeine) to strong opioids such as morphine. For neuropathic pain, that is pain resulting from primary involvement of peripheral or central neural structures treatment begins with anticonvulsant drugs that may reduce neuronal excitability and tricyclic antidepressants. Nonsteroidal anti-inflammatory drugs are often tried but rarely offer substantial relief, and opioid drugs may be considered in severe cases of neuropathic pain but appear to be less effective in the treatment of neuropathic pain than they are in the treatment of inflammatory pain.

Because the currently available drugs act through mechanisms that are not specific to pain-related neural pathways, off-target effects unrelated to the relief of pain often limit the maximum dose of analgesic medication that can be prescribed. In the special case of the potent opioid analgesic morphine, for example, activation of opioid receptors in regions of brain unrelated to pain perception results in somnolence, confusion, and ultimately impaired respiration, while activation of opioid receptors in the gut and the urinary bladder result in nausea, constipation and urinary retention. In addition, continued treatment with opioids results in the development of tolerance leading to a requirement for escalating doses of medication to achieve the same result. Opiate treatment is further complicated by the possibility of drug abuse, unrelated to pain relief.

Inherited monogenic causes of spontaneous pain are fortunately rare, but nonetheless gene transfer to the nervous system has been pursued in an attempt to overcome the limitations of currently available agents. The rationale for the use of gene therapy is based on the observation that the parsimonious use of a limited repertoire of neurotransmitters, receptors, and voltage gated ion channels in the nervous system makes it difficult to selectively target pain-related pathways with systemically administered drugs. In contrast, gene transfer may be used to express short-lived potent bioactive molecules at restricted sites in the nervous system, and thus selectively target nociceptive neurotransmission. Several different strategies have been employed. The most straightforward approach involves the expression of inhibitory neurotransmitters, usually at the spinal level, to block nociceptive neurotransmission at the first synapse between the primary peripheral nociceptor and the second order neuron in the spinal cord. Other investigators have used gene transfer vectors to express anti-inflammatory cytokines in order to block central neuroimmune activation that appears to play an important role in the transition from acute pain perception to the chronic pain state. More recently, vectors coding for antisense or microRNA sequences have been used to reduce the expression of gene products essential to the development of chronic pain.

Transgene-mediated expression of opioid peptides

One important site of action of opioid peptides on nociception is the spinal dorsal horn, where neurotransmission by the primary afferent nociceptor onto second order projection neurons is modulated by opioid peptides released by intrinsic interneurons [3,4]. The first gene transfer approach to delivery of opioid inhibitory neurotransmitters to the spinal cord was reported by Iadarola and colleagues who demonstrated that an adenoviral vector expressing beta-endorphin injected intrathecally would produce endorphin to reduce pain perception in rodents [5]. The recombinant adenovirus vector employed in this study was constructed to lack the tumor-causing gene E1a, and expressed high levels of beta-endorphin from transduced meningeal cells. However, while adenoviral vectors in general show remarkable ability to express transgenes at very high levels, the robust immune response

generated by these vectors [6] has posed a substantial handicap for many proposed applications.

An alternative approach to transgene-mediated expression of opioid peptides was pioneered by Wilson and co-workers, who demonstrated that replication defective herpes simplex virus (HSV) based vector containing the proenkephalin gene, injected subcutaneously in to the paw, would reduce hyperalgesic C-fiber responses [7]. The route of gene delivery using HSV was unique, based on the biology of the parental virus. HSV is a large double stranded DNA virus that in its natural life cycle is spread by contact, infecting and replicating in skin or mucous membrane. Viral particles released in the skin are taken up by sensory nerve terminals and then carried by retrograde axonal transport to the neuronal perikaryon in the DRG, where the wild-type virus may either re-enter the lytic cycle, or establish a latent state characterized by the persistence of viral genomes as a non-replicating intranuclear episomal element. Vectors constructed from HSV that retain the targeting properties of the parental virus are thus efficiently taken up from skin inoculation and delivered to the DRG.

Two approaches have been used to construct recombinant HSV vectors for use in gene transfer. The original vector tested by Wilson et al was deleted for the viral thymidine kinase (*tk*) gene. Because the viral *tk* is required for replication in neurons, recombinant herpes viruses defective in *tk* can be propagated in culture and will replicate in skin, but are unable to replicate in the DRG and are thus forced into a pseudo-latent state. More crippled recombinants have subsequently been constructed. HSV is a complex virus with more than 85 genes coded in the 150 kb genome, but those genes are expressed in a rigidly ordered temporal cascade. Five immediate early (IE) gene products, infected cell polypeptide (ICP) ICP0, ICP4, ICP22, ICP27 and ICP47 are expressed immediately on entry of the genome into the nucleus; RNA for these IE genes are expressed even in the presence of protein synthesis inhibitors. Expression of early genes that code for proteins involved in DNA replication and the late genes that largely code for structural HSV proteins require synthesis of IE gene products [8]. Thus, crippled nonreplicating recombinants can be created by deletions of essential IE genes, such as ICP4, from the viral genome. Such recombinants can be propagated in cells that provide the missing gene product *in-trans*, but are incapable of replication *in vivo* [9]. More recently, vectors have been constructed by deleting two IE genes, ICP4 and ICP27 and modifying the promoters of IE genes ICP22 and ICP47 such that they activate with early transcriptional kinetics, thus effectively preventing the expression of the IE gene products, ICP4, ICP27, ICP22 and ICP47 [10]. These vectors are completely incapable of replication *in vivo*, but because the recombinant particles retain the targeting properties of the wild-type virus can be used to effectively deliver genes from the skin to the sensory ganglion *in vivo*.

Pohl and co-workers showed that a *tk*-defective HSV recombinant can be used to express enkephalin in DRG neurons *in vivo* [11], and subsequently demonstrated that subcutaneous inoculation of the vector reduced pain-related behaviors in a rodent model of polyarthritis induced by injection of complete Freund's adjuvant (CFA) [12]. Interestingly, expression of enkephalin in this model not only reduced pain-related behaviors but also prevented cartilage and bone destruction in the inflamed joints, and effect that appears to result from release of enkephalin from the peripheral sensory terminals in the joint. This inference was supported by two lines of evidence; the effect could be blocked by systemic administration of naloxone-methiodide [12], a substituted analogue of the opioid receptor antagonist that crosses the blood brain barrier poorly, and axonal transport from the transduced cell bodies towards the periphery (as well as towards the spinal cord) could be demonstrated by application of a ligature to the nerve [13].

Similar effects were subsequently demonstrated using a non-replicating HSV vector deleted for both copies of the essential HSV IE gene ICP4. Subcutaneous inoculation of the enkephalin-producing non-replicating vector was shown to produce an analgesic effect in the formalin injection model of inflammatory pain [14]. The effect of vector-mediated enkephalin release was maximal in animals inoculated one week prior to formalin test and waned over the course of the subsequent several weeks. The time course of the analgesic effect of the vector is consistent with the known time course of expression driven by the human cytomegalovirus immediate early promoter (HCMV IEp) employed to drive transgene expression from this vector. Further evidence that the loss of analgesic effect is not a result of tolerance was provided by the observation that reinoculation of a similar dose of the same vector at 4 weeks, after the analgesic effect of the initial inoculation had largely disappeared, was effective in reestablishing the analgesic effect. The observations regarding reinoculation, which have been repeated in several different models of pain and with different transgene products indicates the absence of any significant immune response to vector inoculation in the rodent.

Subsequent work has extended the observations regarding the enkephalin-producing HSV vector to other models of chronic or persistent pain. In the selective spinal nerve ligation model of neuropathic pain, injection of the HSV vector produces an analgesic effect that persists for several weeks and is continuous over time, i.e. stable in magnitude throughout the day. In addition, the vector-mediated analgesic effect was shown to be additive with morphine (reducing the ED₅₀ of co-administered morphine by an order of magnitude), and to persist in animals that are rendered tolerant to the analgesic effects of morphine [15]. Similar to the observations in the formalin test, this enkephalin-expressing HSV vector with gene expression under the control of the HCMV IEp produces an analgesic effect persists several weeks, and the analgesic effect could be reestablished by reinoculation of the vector after the initial effect had waned [15]. In another model of neuropathic pain, the infraorbital nerve constriction model of craniofacial pain, Pohl and colleagues demonstrated that inoculation of the enkephalin-expressing vector in the face produced in a significant reduction in mechanical hypersensitivity on the affected side [16].

The effect of vector-mediated enkephalin production on visceral pain has been examined in two different models of visceral pain. Yoshimura *et al.* showed that following injection into the rat bladder, HSV-mediated enkephalin could be detected in bladder afferent pathways, and that vector-mediated enkephalin effectively attenuated capsaicin-induced bladder irritation and resultant bladder hyperactivity [17,18]. Westlund and colleagues recently reported that in a rodent model of pancreatitis, direct injection of the enkephalin-expressing HSV vector into the pancreas attenuated evoked nocisponsive behaviors [19]. In the latter model, enkephalin production in the pancreas reduced the inflammatory response in a manner similar to that previously demonstrated in polyarthritis [12].

Pain resulting from cancer is complex, with characteristics of both neuropathic and inflammatory types of pain [20]. In a mouse model of bone cancer pain, subcutaneous inoculation of the HSV vector expressing enkephalin resulted in an attenuation of spontaneous nocisponsive behaviors [21].

In addition to studies in rodent models Yeomans *et al.* have demonstrated a similar effect on A-delta and C-fiber mediated responses in macaques following peripheral application of the HSV vector expressing enkephalin to the dorsal surface of the foot [22]. Interestingly, in the primate, the duration of the vector-mediated effect persists substantially longer than the duration that has been observed by other investigators in rodents, suggesting the HCMV IEp may function with different kinetics in primates than in rodents. Taken together, these data provide proof-of-principle that the HSV vector-mediated delivery of enkephalin can provide

an analgesic effect in several different models of pain, and set the stage for a human trial to treat chronic pain using HSV vector-expressed enkephalin (see below).

Enkephalin is the endogenous ligand for the delta opiate receptor in the dorsal horn of spinal cord. In the past decade the endomorphins EM-1 (Tyr-Pro-Trp-Phe-NH₂) and EM-2 (Tyr-Pro-Phe-Phe-NH₂) were identified from bovine brain extracts as highly selective mu receptor agonists and appear to represent the endogenous ligands of the mu receptor [23]. While EM-1-like immunoreactivity is primarily restricted to brain, EM-2-like immunoreactivity is found primarily in the spinal cord and peripheral nervous system and may modulate spinal level pain signaling [24]. Despite the isolation of EM peptides from tissue however, the identity of the gene(s) encoding the precursor protein(s) from which the endomorphin peptides are derived has not been reported, which posed a challenge to gene transfer. Wolfe and colleagues therefore constructed a tripartite synthetic gene cassette with the N terminal signal sequence of human preproenkephalin containing the residues that direct polypeptides into the regulated secretory pathway where proteolytic processing occurs followed by a pair of endomorphin-2 coding elements each flanked by dibasic cleavage sites to provide for processing and peptide liberation by cellular proteases [25]. Each synthetic EM-2 gene was constructed with a C-terminal glycine residue extension so that the product would be processed by the widely distributed enzyme peptidylglycine α -amidating monooxygenase so that processing within the regulated secretory pathway would yield the proper C-terminal amidated peptide.

Processing and release of the authentic peptide *in vitro* was confirmed by HPLC followed by radioimmunoassay and mass spectroscopy [25]. Subcutaneous inoculation of the 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 in the animals. The anti-allodynic effect of vector mediated endomorphin production was blocked by intraperitoneal inoculation of 10 μ g of the highly selective μ -opioid receptor antagonist CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr amide), thus confirming its activity on the mu-opioid receptor *in vivo*.

The effect of HSV-mediated release of endomorphin on inflammatory pain was examined using the complete Freund's adjuvant (CFA) model of inflammatory pain. Inoculation of the vector resulted in a substantial reduction in nocisponsive behaviors correlated with a reduction in peripheral inflammation measured by paw swelling after injection of CFA. The analgesic effect of the vector was blocked by either intraperitoneal (IP) or intrathecal (IT) administration of naloxone methiodide indicating both a central and a peripheral site of action of the released peptide. Endomorphin vector injection also reduced spontaneous pain-related behaviors in the delayed phase of the formalin test. The magnitude of the vector-mediated analgesic effect in that model was similar in naïve animals and in animals with opiate tolerance induced by treatment with morphine 10 mg/kg twice a day. These results suggest that endomorphin released from transfected neurons *in vivo* acts both peripherally and centrally through mu opioid receptors to reduce pain perception.

Transgene mediated release of gamma amino butyric acid (GABA)

Opioid peptides are not the only inhibitory neurotransmitters that are active in the pathways involved in pain perception. Nociceptive neurotransmission is normally under tonic inhibitory control of GABAergic interneurons in the spinal cord, as indicated by the observation that application of GABA blocking agents to the spinal cord results in spontaneous pain related behaviors in normal animals. In addition, recent evidence suggests that the emergence of chronic pain in the setting of peripheral nerve injury is related to the loss of GABAergic tone in the dorsal horn of spinal cord [26], correlating with the common

observation that neuropathic pain relatively refractory to opioid therapy. We constructed a replication-incompetent HSV vector encoding the 67 kD isoform of human glutamic acid decarboxylase (GAD67), the enzyme that decarboxylates glutamic acid to produce GABA [27]. GAD67 vector-transduction of DRG neurons *in-vitro* or *in-vivo* resulted in the expression of GAD67 and the constitutive release of GABA from transduced neurons [28]. Pharmacologic and electrophysiologic evidence suggested that the consecutive release of the neurotransmitter occurs through the action of the GABA transport, functioning in a “reverse” direction because of the high levels of cytoplasmic GABA produced by the transgene product. Subcutaneous inoculation of the GAD-expressing HSV vector resulted in a substantial reduction in mechanical allodynia and thermal hyperalgesia in the selective spinal nerve ligation model of neuropathic pain [29] and a reduction in mechanical allodynia and thermal hyperalgesia in a model of below-level post spinal cord injury pain created by T13 hemisection [27]. The vector-mediated analgesic effect was reversed in part by intrathecal administration of the GABA receptor antagonists, bicuculline or phaclofen, consistent with an effect that is mediated by both GABA(A) and GABA(B) receptors.

The dorsal horn of spinal cord is not the only level in the ascending nociceptive pathway at which targeted gene transfer can be used to modulate nociception. Wilson and colleagues demonstrated almost 10 years ago that bilateral injection into the amygdala of a replication-impaired HSV vector expressing enkephalin results in a selective, naloxone-reversible abolition of phase 2 flinching behavior in the formalin test compared to rats infected with a control virus [30]. Jasmin and colleagues showed that a local increase in GABA in the rostral agranular insular cortex achieved by inoculation of a herpes amplicon vector expressing GAD produced lasting analgesia through by enhancing the descending inhibition of spinal nociceptive neurons [31].

Neurotrophic factors and chronic pain

McMahon *et al.* demonstrated that the direct intrathecal infusion of glial cell-line derived neurotrophic factor (GDNF) reduced ectopic nerve discharges and pain-related behaviors in the partial sciatic nerve injury model of neuropathic pain in rodents [32]. We subsequently showed that a persistent analgesic effect could be achieved by subcutaneous inoculation of a replication defective HSV vector expressing GDNF in the selective L5 spinal nerve ligation of neuropathic pain [33]. HSV-expressed GDNF resulted in a significant anti-allodynic effect, which could be re-established by vector re-inoculation after several weeks without the occurrence of obvious adverse reactions [33].

An alternative vector approach involves the use of lentiviral-based vectors. Retroviruses are RNA viruses that integrate their genome into the host cell by using reverse transcriptase synthesize a DNA copy of the RNA virus genome. The most common retroviruses, oncoretroviruses, were the first viruses engineered to serve as gene transfer vectors, but oncoretrovirus insertion of DNA is largely dependent on cell division so these viruses most efficiently infect dividing cells, which is a major limitation for gene transfer to the nervous system where the target cell populations are terminally differentiated and not dividing. More recently gene transfer vectors have been constructed from modified lentiviruses. The most commonly used system for engineering human lentiviruses is the construction of vector that incorporates elements of lentivirus, an oncoretrovirus, and the envelope components of the vesicular stomatitis virus (VSV-G) into three separate expression plasmids. Co-transduction of cells with these plasmids provides all the structural components to generate non-replicating particles which contained the transgene sequence encompassed within one of the plasmids [34].

Lentiviral vectors expressing GDNF injected unilaterally into the spinal dorsal horn 5 weeks before a spinal nerve ligation results sustained expression of transgenes in both neurons and glial cells, induces a significant reduction of ATF-3 up-regulation and IB4 down-regulation in damaged DRG neurons, and produces a partial but significant reversal of thermal and mechanical hyperalgesia spinal nerve ligation [35]. Eaton and co-workers demonstrated similar results using an adenoassociated viral vector engineered to express BDNF injected into the dorsal horn of spinal cord [36]. Adenoassociated viral vectors expressing the mu opioid receptor and injected directly into dorsal root ganglia potentiate the effects of systemically administered opioids in models of chronic pain [37]. While these approaches have substantial advantages for the study of pain-related mechanisms in the dorsal horn and DRG, they are unlikely to be developed into a platform for gene therapy of pain in patients because of the invasive nature of the approach.

Gene transfer to modulate the neuroimmune response in chronic pain

There is substantial evidence that the development of chronic pain involves activation of spinal cord glia with consequent release of pro-inflammatory cytokines [38-41]. Among the released pro-inflammatory cytokines, tumor necrosis factor alpha (TNF α) plays a central role in the induction of chronic inflammation and pain by binding to the TNF α receptor and promoting a nuclear factor kappa B dependent transcriptional production of proinflammatory cytokines such as interleukin-1beta (IL-1 β) [42,43]. Others have shown neutralizing antibodies directed against the p55 TNF receptor (TNFR) reduce thermal hyperalgesia and mechanical allodynia, while the intrathecal administration of the recombinant p75 soluble TNFR (sTNFR) peptide (etanercept) reduces mechanical allodynia in a rat model of neuropathic pain [44-46]. We used an HSV vector-mediated transfer of a gene coding for the p55 sTNFR antagonist of TNF α to DRG neurons [47]. Following subcutaneous vector inoculation, HSV-expressed p55 sTNFR is released into the dorsal horn of spinal cord in rats with spinal nerve ligation induced neuropathic pain. Vector-mediated release of p55 sTNFR in spinal cord reduced the behavioral manifestations of neuropathic pain while simultaneously reducing the phosphorylation of p38 α , the expression of IL-1 β and the expression of membrane-associated TNF α (mTNF α) in the spinal cord. These results implicate a unique mechanism by which p55 sTNFR can downregulate TNF α and IL-1 β expression by reverse signaling through mTNF α . Similar results were found in a separate study using a model of below-level central neuropathic pain following thoracic spinal cord hemisection [48].

Interleukin-4 (IL-4) is a prototypical anti-inflammatory cytokine [49,50]. Furlan *et al.* and Poliani *et al.* had shown that HSV-expressed IL-4 prevents the development of autoimmune encephalitis in Biozzi AB/H mice and in rhesus monkeys [51]. We examined the analgesic effects of locally delivered IL-4 in neuropathic pain by using a replication defective HSV vector to express the coding sequence for IL-4 under the transcriptional control of the HSV ICP4 immediate early promoter [52]. Vector-mediated expression of IL-4 did not alter either the paw withdrawal latency to thermal stimuli or tactile threshold in normal animals but reduced mechanical allodynia and reversed thermal hyperalgesia in a rat model of SNL. In addition, the development of thermal hyperalgesia and tactile allodynia were delayed when the vector was inoculated one week before SNL. Results from this study show that HSV-mediated expression of IL-4 effectively reduces the aspects of neuropathic pain-related behavior and can potentially be used as an analgesic in gene therapy-based approaches to treat chronic pain.

An alternative approach to spinal delivery of IL-10 has been pioneered by Watkins and co-workers. In rodent experiments, the Watkins group has demonstrated that adenovirus, adenoassociated virus, or simple plasmids encapsulated in liposomes and constructed to

express IL-10, injected intrathecally result in release of IL-10 into the cerebrospinal fluid. Transgene-mediated expression of the anti-inflammatory cytokine can prevent the development of neuropathic pain or reverse established neuropathic pain, in models of neuropathic pain caused either by nerve constriction or injection of algogenic substances into the nerve sheath [53-55]. The reduction in pain-related behaviors is usually long-lasting, although for reasons that are not entirely clear, a pair of injections just a few days apart appears to be much more effective than a single injection of the gene transfer vector.

HSV vectors to reduce expression of endogenous genes in the dorsal root ganglion

All of the studies discussed above have used gene transfer to express potentially analgesic peptides at specific sites within the nervous system. Recent advances in RNA technology have made it possible to knock down endogenous gene expression *in vivo*, thus allowing investigators potentially to modulate the expression of genes whose products might contribute to the perception or persistence of pain. Among the gene products expressed in primary nociceptors, the voltage-gated sodium channel isoform Nav1.7 and the peptide neurotransmitter calcitonin gene related peptide (CGRP) are two gene products that experimental evidence indicates may play an important role in pain perception. Yeomans, Wilson and colleagues have reported that an HSV vector coding a sequence antisense to Nav1.7, applied to the skin prevents the increase in Nav1.7 expression caused by CFA-induced inflammation in transduced DRG neurons and that this effect correlated with a reduction in the development of hyperalgesia in both C and A-delta thermnociceptive tests [56]. More recently, the same group has shown that an HSV vector coding a sequence antisense to CGRP reduces CGRP expression in transduced DRG neurons with concomitant reduction in nociceptive neurotransmission *in-vivo* [57].

Conclusions and Future Directions

The preclinical studies reviewed above provide proof-of-principle for the potential utility of HSV-based vectors in the treatment of chronic pain. In the treatment of glioblastoma, replication compromised HSV vectors injected directly into brain in a number of phase 1 and phase 2 trials have not demonstrated significant toxicity [58,59], so cutaneous application of a non-replicating HSV vector should not pose a serious problem, though the first phase 1 safety and dose finding trial is yet to start. The animal studies suggest that several different gene products could be delivered by gene transfer to provide an analgesic effect, and it is possible that the choice of gene product may depend on the type of pain being treated. The first phase 1 human trial of HSV will test the well-characterized enkephalin-expressing vector, with the availability of naloxone reversal providing an additional element of safety.

An important issue that will need to be addressed regards vector delivery. In rodent models vector delivery from the skin has been achieved by application to depilated skin [7,56] or by subcutaneous injection into the footpad but the structure and innervation of human skin is quite different from that of the rodent, and vector delivery will need to be optimized if biologically meaningful effects are to be achieved. Nonetheless, the studies to date provide encouragement that gene transfer may ultimately prove to be useful in the treatment of chronic pain.

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