MAYO CLINIC

Future Directions in Pain Management: Integrating Anatomically Selective Delivery Techniques With Novel Molecularly Selective Agents

Josef Pleticha, MD; Timothy P. Maus, MD; and Andreas S. Beutler, MD

From the Departments of Anesthesiology and Oncology (J.P., A.S.B.) and Radiology (T.P.M.), Mayo Clinic, Rochester, MN.

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Learning Objectives: On completion of this article, you should be able to (1) recognize the health care and societal cost of chronic locoregional pain; (2) define the 2 paradigms for analgesic drug development, conventional drug discovery, and gene therapy; (3) list novel analgesic targets explored by gene therapy; and (4) discuss the role of interventional drug delivery for development of gene therapy for chronic locoregional pain.

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Abstract

Treatment for chronic, locoregional pain ranks among the most prevalent unmet medical needs. The failure of systemic analgesic drugs, such as opioids, is often due to their off-target toxicity, development of tolerance, and abuse potential. Interventional pain procedures provide target specificity but lack pharmacologically selective agents with long-term efficacy. Gene therapy vectors are a new tool for the development of molecularly selective pain therapies, which have already been proved to provide durable analgesia in preclinical models. Taken together, advances in image-guided delivery and gene therapy may lead to a new class of dual selective analgesic treatments integrating the molecular selectivity of analgesic genes with the anatomic selectivity of interventional delivery techniques.

 ■ Mayo Clin Proc. 2016;91(4):522-533

Chronic pain affects more than 116 million Americans and is one of the leading causes of medical appointments and disability, as reported by the Institute of Medicine in 2011.[1,2] Overall, the annual cost of chronic pain is estimated to be between $560 and $635 billion in the United States alone.[1] Although chronic pain is often viewed as a systemic disorder, the data provided by this report show that most patients experience locoregional pain, ie, pain syndromes anatomically limited to a sensory field of 1 or only a few peripheral or spinal nerves.

The Institute of Medicine identified locoregional musculoskeletal pain as the leading presentation of chronic pain in the United States. Specifically, the 2 most prevalent causes of chronic pain in the population were low back pain and knee pain, as documented by a survey performed by the National Center for Health Statistics, whereby 28.1% of adult Americans reported low back pain and 19.5% localized knee pain during the past 3 months.[1] Locoregional pain is also responsible for a substantial portion of the financial burden of chronic pain. Low back pain is estimated to cost $30 billion in direct health care expenditures and $100 to $200 billion in decreased wages and disability in the United States annually.[3,4] As for arthritic pain, annual direct health care expenses are estimated to approach $81 billion, with indirect costs reaching $189 billion annually.[5]

Similar to musculoskeletal pain, cancer pain—a subset of chronic pain that is particularly challenging to manage—also frequently presents as a locoregional syndrome. Fifteen percent of patients with cancer pain experience anatomically localized pain, such as pain originating from bone metastases or neuropathic pain from nerve compression or invasion.[6] Pain in these patients often cannot be adequately controlled by systemic analgesic treatments because the high doses needed for analgesia trigger intolerable adverse effects.[7] Although the best short-term outcomes in these patients are, therefore, frequently achieved by regional administration of local anesthetics or by selective neurolysis, neither approach is durable.[6]

THE SEARCH FOR SYSTEMIC ANALGESIC THERAPIES

The Conventional Drug Discovery Paradigm

Current analgesic therapies fail in a substantial number of patients with chronic pain.[6] This failure is most striking in cancer pain, where pain remains inadequately controlled in 50% of all patients.[8] Despite the search for novel analgesic treatments during the past few decades, currently available analgesic drugs exert their effect through very few categories of molecular targets.[9,10] Recent advances in neurobiology of nociception, however, identified a variety of candidate therapeutic targets located in the peripheral nervous system that are not used by current analgesic drugs. Important examples include ion channels expressed by primary sensory neurons, cytokines that play a critical role in the pathogenesis of neuropathic pain, and several inhibitory and excitatory neurotransmitters that modulate nociceptive signaling.

Characterization of these nociceptive mechanisms led to a search for a new generation of analgesic drugs using the techniques of conventional drug discovery (CDD); CDD, ie, the search for new small-molecule therapeutic entities, largely relies on empirical screening of candidate compounds. Two approaches are used by the pharmaceutical industry and academic laboratories involved in CDD. First, phenotype-directed screening seeks to identify chemical entities that have a desirable effect on the phenotype of a disease model without previous knowledge of their molecular mechanism of action.[11] Various assays modeling the disease phenotype on the level of a cell or an organism have been described.[12] Efficacious compounds are then examined for molecular mechanism of action using reverse biology techniques. Second, target-directed screening relies on biochemical knowledge of specific molecular targets and uses small-molecule screening strategies, typically by examining large libraries of compounds.[13] The actual biologic effect of thus discovered compounds is then tested in vivo in disease models. Although there has been a substantial effort to facilitate CDD by using high-throughput assays and by implementing genomic data, the discovery of new small-molecule drugs is declining in all medical fields. In fact, pharmaceutical industry data show that

the number of truly innovative drugs (defined as new molecular entities) approved by the major regulatory bodies (such as the US Food and Drug Administration) has been decreasing by 50% every 5 years worldwide.[14-16]

Although multiple candidate analgesic agents that had been identified through CDD were tested in preclinical models, the success regarding clinical translation has been very limited, as reviewed next.

Voltage-Gated Sodium Channels

Voltage-gated sodium channels (Na_v) are responsible for the formation of action potentials in all excitatory cells. Several Na_v subtypes are expressed by primary sensory neurons but not by other excitatory cells, such as neurons of the central nervous system (CNS) or myocytes.[17] Specifically, $Na_v1.3$, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ have been found to be involved in nociception. All of these channels undergo upregulation in rodent models of nerve injury or inflammatory and neuropathic pain.[17] In addition, mutations in genes encoding these channels are associated with inherited pain disorders in humans. Gain-of-function mutations of $Na_v1.7$ result in inherited erythromelalgia, an autosomal dominant disorder manifesting by extreme heat hyperalgesia.[18] Although clinical manifestations of gain-of-function and loss-of-function mutations in $Na_v1.3$ and $Na_v1.8$ are less clearly defined, they have also been associated with the development of painful neuropathies and insensitivity to pain, respectively, in humans.[19]

A variety of molecular entities have been tested in the preclinical setting that selectively inactivate Na_v, including toxins isolated from tarantula venom or small-molecule compounds identified via target-directed screening.[20-23] Yet, despite an ongoing effort, none of these agents have been approved as a drug for use in humans.

Voltage-Gated Calcium Channels

Similar to Na_v, voltage-gated calcium channels (Ca_v) expressed in primary sensory neurons result in an increased firing frequency.[24] At the level of the spinal cord, the upregulation of Ca_v secondary to nerve injury has been described in rodent models of neuropathic pain.[25] Specifically, $Ca_v2.2$ seems to play a critical role in neuropathic pain. Inactivation of this channel by channel-binding peptide 3 inhibits trafficking of $Ca_v2.2$ to the cytoplasmic membrane and, thereby, diminishes membrane excitability of nociceptive neurons, resulting in attenuation of nocifensive behavior in rodent models.[26]

Gabapentinoids are the only class of systemic analgesic drugs that may exert their therapeutic effect through interacting with Ca_v. Gabapentinoids were initially conceived as orally available analogues of the γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, for the treatment of seizure disorders.[27] Although the antiepileptic efficacy of gabapentinoids did not hold up to the initial promise, they have been found to be efficacious for the treatment of chronic neuropathic pain.[28,29] Although the mechanism of action of gabapentinoids has not been fully elucidated, they have been found to bind to the α2δ-1 subunit of the Ca_v, and it is, therefore, thought that they may, at least in part, exert analgesia through inhibition of Ca_v.[30]

A search for selective and potent Ca_v antagonists identified ω-conotoxin MVIIA, a neurotoxic peptide isolated in the 1980s from the venom of marine snails belonging to the genus *Conus*.[31,32] ω-Conotoxin MVIIA selectively blocks N-type Ca_v present in nociceptive fibers. Despite efforts over 2 decades, a search for a drug with the mechanism of action analogous to ω-conotoxin MVIIA that would be efficacious and nontoxic after systemic administration has failed. Only in 2004, ziconotide, a ω-conotoxin MVIIA derivative, was approved for localized intrathecal (IT) delivery in patients with severe, otherwise intractable pain.[33]

Voltage-Gated Potassium Channels

Voltage-gated potassium channels (K_v) are also critical determinants of the firing frequency, and their downregulation results in membrane hyperexcitability.[17,34] Although several potassium channels are expressed by primary nociceptive fibers, $K_v1.2$ has been most thoroughly studied in association with chronic pain and has been found to be markedly downregulated in the dorsal root ganglion (DRG) neurons in the setting of nerve injury and neuropathic pain.[35] Yet, no analgesic drugs that modulate nociceptive signaling through K_v are clinically available.

Mayo Clin Proc. ■ April 2016;91(4):522-533 ■ http://dx.doi.org/10.1016/j.mayocp.2016.02.015
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Ligand-Gated Cation Channels

Ligand-gated ion channels are an attractive target for drug development because their endogenous ligands can serve as a molecular pattern for discovery of compounds with analogous pharmacodynamics but therapeutically more favorable pharmacokinetics. The *N*-methyl-D-aspartate (NMDA) glutamate receptor mediates long-term potentiation, which has been found to play a key role in the development of chronic pain.[36,37] The NMDA receptor antagonist ketamine, a dissociative general anesthetic agent, is used in certain subgroups of chronic pain, but data on its efficacy are inconclusive.[38-42] Despite an ongoing research effort, no clinically available NMDA antagonist more specific to nociception has been found.

Transient receptor potential channels (TRP) constitute another family of ligand-gated cation channels. Several TRP are expressed by the primary sensory neurons and are typically activated by noxious stimuli.[43] TRP, subfamily V, member 1 (TRPV1) is a particularly attractive target for drug development because it is almost exclusively expressed by primary nociceptive neurons and its inactivation results in loss of pain and heat sensation while other sensory modalities remain intact.[44,45] Capsaicin, the pungent substance in chili peppers, is the only analgesic agent acting through TRPV1 clinically available to date and is used in the form of a patch to treat locoregional pain.[46] Capsaicin is a reversible TRPV1 agonist and is thought to induce analgesia by desensitizing TRPV1+ nerve fibers.[47] A more intuitive approach to using the TRPV1 pathway is the use of TRPV1 receptor antagonists. Although a variety of TRPV1 antagonists have been tested in preclinical models, none have been successfully translated to the clinical setting owing to their off-target adverse effects, which included hyperthermia and impaired noxious heat sensation, after systemic delivery.[48]

Anti-inflammatory Cytokines

Glial cells have been reported to initiate and maintain neurogenic inflammation in the setting of chronic neuropathic pain, resulting in increased neuronal excitability.[49] The effect of glia on the primary sensory neurons may be mediated by upregulation of proinflammatory cytokines, including tumor necrosis factor, interleukin 1β, and various chemokines.[50] The proinflammatory and pronociceptive effect of these cytokines is countered by anti-inflammatory cytokines. The principal anti-inflammatory cytokine is interleukin 10 (IL-10). Therefore, IL-10 is an attractive agent to treat neuropathic pain, which is known to be relatively refractory to opioid analgesics.

The IT administration of recombinant IL-10 has, indeed, been found to reduce neuroinflammation and allodynia in animal models of neuropathic pain.[51,52] However, direct delivery of the recombinant IL-10 was found not to be feasible for clinical translation because of its high cost, its inability to cross the blood-brain barrier, and its half-life of only 2 hours in the cerebrospinal fluid after IT delivery.[53,54]

Pain-Modulating Neurotransmitters

Development and maintenance of chronic pain states is associated with alteration of the neurotransmitter milieu in the CNS.[55] The neuroinhibitory transmitter GABA is known to inhibit neuropathic pain signaling on the level of the dorsal horn of the spinal cord.[56] Although 2 classes of GABAergic drugs—baclofen and benzodiazepines—are used in clinical practice in select patients, their effect is not selective to the nociceptive pathways, resulting in a very low therapeutic index in the context of pain management.[57] Despite substantial effort, novel and more selective analgesic agents using the GABAergic pathway are not clinically available.

Another neurotransmitter involved in pain signaling is substance P. Substance P is produced by neurons throughout the peripheral nervous system and CNS, and it exerts its effect by activating neurokinin type 1 (NK_1) receptor.[58] Studies using NK_1 receptor knockout mice and NK_1 receptor antagonists in rodents suggested that blocking substance P signaling would result in profound analgesia, thereby generating widespread enthusiasm regarding clinical translation.[59,60] However, clinical studies of several NK_1 receptor antagonists given by the systemic route reported an almost complete lack of analgesic efficacy in humans.[61] The failure of NK_1 receptor antagonists may have been considered a breaking point in analgesic drug discovery and development as it brought the realization that there may be no magic bullet consisting of

a chemical compound targeting a single nociceptive mechanism with a degree of nociceptive specificity that could rival (or exceed) the analgesic activity of opioids.

LOCOREGIONAL ANALGESICS: AN ALTERNATIVE APPROACH TO DRUG DEVELOPMENT

Clinical Interventional Pain Procedures for Locoregional Pain Syndromes

Interventional drug delivery for locoregional chronic pain is one of the most common procedures performed in clinical practice.[62] Multiple techniques that target various pain generators under imaging guidance are used.[63] The most frequent targets for interventional drug delivery are the spinal nerves, which are accessed either IT or via the epidural (ED) route under fluoroscopic guidance. More than 2.5 million ED injections were performed in 2013 in the United States.[64] Although all these procedures are remarkably safe, their efficacy has recently come under scrutiny.[65] For the ED injection, there is evidence of moderate efficacy for some pain syndromes, such as lumbar radiculitis, but no evidence of efficacy in others, such as spinal stenosis (neurogenic intermittent claudication).[66]

The chief reason for the limited efficacy of interventional procedures for chronic pain is the small repertoire of analgesic agents available for these routes of delivery. For IT delivery, the available drugs are limited to opioids, benzodiazepines, baclofen, ziconotide, local anesthetics, and ketamine.[57] For ED delivery, local anesthetics and corticosteroids constitute the only agents available to date. The pharmacologic effect of these agents is relatively nonspecific, and its duration is only transient in most patients, requiring either implantation of a pump for the IT route or repeated interventions for the ED route; on average patients receive 2 to 3 injections of ED corticosteroids per year.[64]

Although the discovery of novel analgesic agents specifically designed for anatomically selective routes of delivery, such as ED, is a major unmet clinical need, this area has not been appreciably explored by the pharmaceutical industry and research laboratories. The search for novel molecular targets has focused on systemically administered drugs, whereas drug development for localized delivery mainly involved optimizing the formulation of already available agents for ED delivery, such as depot liposomal forms of corticosteroids extending their biological half-life (currently in the phase of preclinical development).[67] It is notable that although the field of CDD focused on systemic analgesic drugs, the major truly innovative agent recently approved for the treatment of chronic pain, ziconotide, is a locally delivered (IT) agent.

Requirements for a New Generation of Analgesic Drugs for Interventional Delivery

A desirable agent for localized, interventional delivery would ideally meet 2 requirements that differ from systemic drugs developed via CCD: (1) prolonged or even permanent activity after a single administration and (2) ability to penetrate the target tissue locally without a need for sufficient bioavailability after systemic administration and for lack of off-target toxicity. Although the first requirement raises a conceptual bar that the candidate interventional agents need to reach compared with most systemic drugs currently under development via CDD, the second requirement lowers this bar substantially. The combination of these 2 requirements, ie, the need for long-term effect after a single administration and lessened concern for off-target distribution and toxicity, seems to be uniquely suited for an emerging drug discovery and disease treatment paradigm: gene therapy.

GENE THERAPY

Beyond Hereditary Disorders

Gene therapy was initially developed to correct dysfunctional genes in rare monogenic disorders, such as inborn errors of metabolism or blood clotting disorders.[68] However, the greatest potential impact of gene therapy may lie in the treatment of common, acquired disorders.[69,70] The field of gene therapy reached critical benchmarks during the past several years.[71,72] In 2012, alipogene tiparvovec became the first gene therapy product to be commercially approved in Europe (for the treatment of lipoprotein lipase deficiency).[73] In 2015, talimogene laherparepvec was the first gene vector brought to market in the United States (for the treatment of inoperable malignant melanoma and head and neck cancer).[74,75]

Mayo Clin Proc. ■ April 2016;91(4):522-533 ■ http://dx.doi.org/10.1016/j.mayocp.2016.02.015
www.mayoclinicproceedings.org

Gene Vectors: A Distinct Class of Therapeutic Agents

In gene therapy, a vector serves as an intracellular delivery mechanism for the instruction set provided by the therapeutic gene (transgene) to the targeted cells.[76] The encoded proteins or peptides are expressed by the target cells, thereby harnessing their machinery to produce the therapeutic substance locally. Vectors are the most commonly genetically engineered viruses whose ability to replicate is artificially disrupted and part or all of their genome is replaced by a transgene of choice.[77] Unique from other drug constructs, the viral vector—transgene package is modular. The surface characteristics of the viral shell (capsid serotype) will determine its tissue selectivity (tropism), and this can be varied independently of the carried transgene. This provides great flexibility in testing large numbers of potential transgene products directed at varying molecular targets. This approach also enables overexpressing, silencing, or de novo expressing of virtually any gene, thereby affecting any signaling pathway. A further variable that can be introduced is selective anatomic delivery, which may augment or overcome the challenges of insufficient tissue tropism.

Primary Sensory Neurons as the Target of Gene Therapy for Pain

The primary sensory neurons, as the point of aggregation of nociceptive signaling from the periphery, are a rational target of gene therapy.[78] These neurons reside in the DRG, a structure that is anatomically accessible, obviating the risk of CNS instrumentation. There are also appropriate small-animal models of DRG access and peripheral nociceptive testing.[79,80] These characteristics have focused attention on DRG neurons as the primary target for modulation of nociception. Gene therapy may be directed to DRG neurons either by transducing them directly or by transducing anatomically or functionally related structures, such as the glia or the meninges, whereby nociception is indirectly modulated by their interaction with DRG neurons.

Targeting the Primary Sensory Neurons by Gene Vectors

To attain gene transfer to the primary sensory neurons, several routes of vector delivery have been explored. First, replication-incompetent herpes simplex virus (HSV) vectors delivered by skin inoculation were pioneered by Fink, Glorioso, and others.[81,82] This approach takes advantage of the inherent ability of this virus to undergo retrograde axonal transport from the sensory nerve terminal to the cell body and, thereby, to transduce DRG neurons whose dendrites originate in the injected sensory field of the skin. Although this selectivity makes HSV attractive for gene transfer to the peripheral nervous system, it also restricts its utility for other gene therapy applications; the overall translational experience with this vector remains, therefore, relatively limited.

Second, IT injection of several serotypes of adeno-associated virus (AAV) vectors was found to selectively transduce the primary sensory neurons.[83-87] Adeno-associated virus is the leading vector type across many gene therapy fields because it is not associated with any disease in any species, is devoid of any viral genes, and yields stable gene expression for months to years.[88] The initial safety concerns regarding AAV vectors, which included insertional mutagenesis, germline transmission, replication escape, and dissemination of the vector to the public domain, have been thoroughly addressed in the field and do not seem to be applicable in the context of human trials, as previously reviewed in detail elsewhere.[69] Furthermore, the difficulties with large-scale manufacturing of AAV vectors, which were holding back the clinical translation of AAV-based gene therapy, have recently been overcome.[89] A relative restriction associated with AAV is the need to switch capsids if serial treatments are needed because serologic immunity renders the readministration of a vector with the same serotypes ineffective.[90] In addition to AAV, IT delivery of other vector types, such lentiviruses or AAV plasmid DNA, was also tested for gene therapy for pain. Although efficacious in certain therapeutic strategies, these vectors did not transduce the primary sensory neurons but rather glial cells or the meninges.[91]

Third, to further improve the efficiency of gene transfer, several groups have targeted the nociceptive neurons directly by intraganglionic injection of AAV in rodents.[92-94] Although this approach led to highly efficacious and selective gene transfer to the DRGs in a rodent model, an open surgical procedure consisting of paravertebral exposure and foraminotomy was required.

MOLECULAR TARGETS OF GENE THERAPY FOR PAIN

The molecular selectivity of gene therapy has been of great interest because it enables utilization of antinociceptive mechanisms that have not been accessible by conventional agents. A variety of approaches have been found to be efficacious in animal models of chronic pain. Important examples of the molecular targets used to date by gene therapy for pain are (1) spinal opioid delivery, (2) anti-inflammatory cytokines, and (3) ion channels and neurotrophic growth factor.

Spinal Opioid Delivery

The opioid system is an attractive target for gene therapy because of the robust clinical experience with exogenous opioids. Although opioids modulate pain processing at multiple levels of the nervous system, delivery of exogenous opioids to the spinal IT space is sufficient to achieve analgesia.[95] Gene transfer of opioid genes selectively to DRG neurons has been found to reduce chronic pain in animal models of locoregional pain (as reviewed next). This approach may eliminate the toxicity of exogenously administered opioids, which is attributed to their extraspinal effects in the CNS and in peripheral tissues.[96-103]

Two principal strategies using opioid signaling for analgesia by gene therapy have been described. First, several groups have used transgenes encoding endogenous opioid agonists delivered to the DRGs. Goss et al[104] used preproenkephalin, a δ-opioid receptor agonist, and endomorphine-2, a μ-opioid receptor agonist, delivered by HSV vectors. We used AAV to deliver prepro-ß-endorphin, an artificial opioid precursor of the μ-opioid agonist peptide ß-endorphin that is engineered to be secreted in a stimulus-independent manner via the constitutive secretory pathway.[105] Administration of these therapeutic genes resulted in robust reduction of allodynia or hyperalgesia in multiple rodent models of chronic neuropathic and inflammatory pain.[84,85,104,106] The second strategy to harness the opioid system for gene therapy of pain consists of transferring genes that encode an opioid receptor. Xu et al[107,108] reported this approach several years ago, but no recent follow-up studies seem to have been attempted. Another approach testing an opioid receptor mutant was reported more recently.[109]

Anti-inflammatory Cytokines

Gene transfer of IL-10 by several vector systems, including HSV and AAV, has been extensively tested in rodent models of chronic neuropathic pain.[84,85,110,111] Production of IL-10 resulted in marked reduction of allodynia and hyperalgesia, a finding verified independently by multiple groups. In addition to IL-10, a tumor necrosis factor—soluble receptor delivered via HSV-mediated gene transfer reduced allodynia in a rodent model of varicella zoster virus—induced pain.[112]

Ion Channels

Discovery of the voltage- and ligand-gated ion channels that are specific to nociception and the failure to generate corresponding conventional analgesic drugs has prompted an effort to use these targets via gene therapy. Two approaches have been used: (1) overexpression of ion channels responsible for membrane hyperpolarization by delivering vectors that carry the gene encoding for the respective channel and (2) downregulation of ion channels responsible for membrane depolarization by RNA interference, ie, by delivering vectors encoding small interfering RNA or small hairpin RNA (shRNA) complementary to the sequence of the respective ion channel, or by gene transfer of genes whose products deactivate these channels.

In the first approach, AAV-mediated gene transfer of $K_v1.2$ in a rat model of neuropathic pain resulted in normalization of $K_v1.2$ expression that had been downregulated in the context of nerve injury. This was associated with a decrease in allodynia in the treated animals.[112] Similarly, overexpression of the glycine receptor (a ligand-gated chloride channel) in the sensory fibers mediated by HSV resulted in attenuation of hyperalgesia in rodent models of neuropathic pain.[113]

In the second approach, Samad et al[114] found that knockout of $Na_v1.3$ by shRNA encoded by an AAV vector delivered into the DRGs attenuated allodynia in the experimental animals. Similarly, knockout of the NR subunit of the NMDA receptor in the dorsal horn of the spinal cord via AAV-mediated transfer of the respective small interfering RNA reduced spinal NMDA currents as well as nociceptive behavior in a model of chronic inflammatory pain.[115,116] Downregulation of the TRPV1 signaling via gene transfer of

Mayo Clin Proc. ■ April 2016;91(4):522-533 ■ http://dx.doi.org/10.1016/j.mayocp.2016.02.015
www.mayoclinicproceedings.org

either shRNA delivered by IT AAV or the dominant negative (poreless) form of the protein delivered by HSV has been found to reduce allodynia in rat models of neuropathic pain.[117-119] Fischer et al[120] downregulated $Ca_v2.2$ by AAV-mediated gene transfer of Ca^{2+} channel-binding peptide 3, also resulting in attenuation of neuropathic pain in the rat model. Ma et al[121] found that overexpression of Kir2.1, an inwardly rectifying potassium channel, delivered by an adenoviral vector into the DRGs suppresses neuronal excitability and prevents the development of hyperalgesia in rodents.

Neurotransmitters and Growth Factors

Delivery of AAV or HSV vectors encoding several isoforms of the GABA gene showed reduction of allodynia in rodent models of neuropathic pain.[122,123] The AAV- or HSV-mediated gene transfer of several neurotrophic growth factors has been investigated as a novel approach to treat chronic pain by reversing the synaptic remodeling described in chronic pain states. This approach could be tested only by gene therapy because conventional pharmacotherapy could not attain target-specific, long-term delivery of an active agent. Several neurotrophic factors, such as brain-derived neurotrophic factor, erythropoietin, glial cell—derived neurotrophic factor, and vascular endothelial growth factor, were found to reduce pain behavior in rodent models of chronic neuropathic pain.[124-127]

Gene Editing

Gene editing is a technique for highly targeted deletion, insertion, or substitution of a desired sequence of a genome.[128] Although multiple gene editing techniques have been described, the field underwent a breakthrough in 2012 when Barrangou, Doudna, Jinek, and others described the clustered regularly interspaced short palindromic repeats (CRISPR) in conjunction with CRISPR-associated protein 9 (CRISPR/Cas9).[128-131] CRISPR/Cas9 allows precise and cost-effective gene editing in vivo, and is poised to revolutionize gene therapy. It seems conceivable that in vivo gene editing using CRISPR/Cas9 delivered by gene vector may give rise to a whole new field of pain therapy.

Chemogenetics and Optogenetics

Chemogenetics and optogenetics represent 2 emerging approaches to selectively and, unlike traditional gene therapy, reversibly manipulate cell phenotypes via gene transfer.[132] Chemogenetics is based on designer receptors exclusively activated by designer drugs (DREADDs). The DREADDs are G protein—coupled receptors artificially engineered to respond to nanomolar concentrations of otherwise biologically inert ligands, such as clozapine-N-oxide.[133,134] Preliminary studies in rodents suggest that selective expression of DREADDs in nociceptive neurons via gene transfer might allow efficient and highly precise exogenous control of nociceptive signaling.[55,135,136] Analogously, optogenetics enables tunable manipulation of cell phenotypes using light.[137] Iyer et al[138] found that AAV-mediated gene transfer of eNpHR3.0, a chloride channel activated by yellow light, to the DRG sensory neurons results in reduction of allodynia in rodent models that is tunable by illumination by light of the respective wavelength.

Pathways to Clinical Translation

Herpes simplex virus encoding preproenkephalin, administered as a series of intradermal injections for intractable locoregional pain caused by cancer, is the only vector so far tested in humans in the context of gene therapy for pain.[139] A phase 1 clinical trial reported safety of the treatment, but the lack of a blinded control group, a design typical of a phase 1 trial, did not allow any conclusions regarding efficacy. A subsequent blinded, placebo-controlled phase 2 trial was initiated in 2011 and completed in November 2013 (according to ClinicalTrials.gov).[140] Of note, HSV was translated from rodent studies directly into clinical trials without testing the approach in a large-animal model as an intermediate step. Should the clinical trial fail to find analgesic efficacy, it may be difficult to discern whether the HSV gene transfer technology or the therapeutic gene was the cause of failure. In such a situation, only large-animal studies may be able to further the understanding.

Adeno-associated virus—based gene therapy has not been tested in patients with chronic pain but in numerous other clinical settings, including phase 1, 2, and 3 trials for a large variety of neurologic and nonneurologic therapeutic applications.[141] In the field of AAV for pain, we translated the approach into large-animal models, first reporting differences in gene transfer

outcomes compared with rodents when the IT route was tested.[142] Subsequently, we developed a method of locoregional delivery of AAV to DRG neurons in a large-animal (swine) model using computed tomography fluoroscopy.[143] This study recreated the gene transfer outcomes of the original rodent studies of intraganglionic AAV delivery while introducing an image-guided intervention technique and thereby obviating the need for open surgical exposure of the DRG that was used in rodents. Examination of the comparative anatomy of porcine and human spines indicates that similar, minimally invasive intraganglionic injection could also be performed in the clinical setting.

CONCLUSION

Interventional pain specialists have a unique skill set to translate gene therapy–based treatment approaches into novel clinical treatments for locoregional pain. Future clinical progress may be driven not only by the rapid advances in gene vector design and clinical availability but also by advances in interventional delivery techniques. Image guidance available for clinical use to date can provide anatomic selectivity for gene vector delivery to select DRGs. Combining the molecular selectivity of gene vectors with the anatomic selectivity of image-guided delivery may spur a new era of interventions for pain using a dual selective approach to analgesic treatment, thereby offering novel treatment options for patients with hitherto intractable locoregional pain.

Abbreviations and Acronyms: **AAV** = adeno-associated virus; **Ca_v** = voltage-gated calcium channel; **CCD** = conventional drug discovery; **CNS** = central nervous system; **CRISPR/Cas9** = clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; **DRG** = dorsal root ganglion; **DREADD** = designer receptors activated by designer drugs; **ED** = epidural; **GABA** = γ-aminobutyric acid; **HSV** = herpes simplex virus; **IL-10** = interleukin 10; **IT** = intrathecal; **K_v** = voltage-gated potassium channel; **Na_v** = voltage-gated sodium channel; **NK_1** = neurokinin type 1; **NMDA** = *N*-methyl-D-aspartate; **shRNA** = small hairpin RNA; **TRP** = transient receptor potential channel; **TRPV1** = transient receptor potential channel, subfamily V, member 1

Correspondence: Address to Timothy P. Maus, MD, Department of Radiology, Mayo Clinic, 200 First St SW, Rochester, MN 55905; and Andreas S. Beutler, MD, Department of Anesthesiology and Oncology, Mayo Clinic, 200 First St SW, Rochester, MN 55905. Individual reprints of this article and a bound reprint of the entire Symposium on Pain Medicine will be available for purchase from our website www.mayoclinicproceedings.org.

The Symposium on Pain Medicine will continue in an upcoming issue.

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