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Spasticity

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REVIEW & ANALYSIS

Motor Neuron Inhibition–Based Gene Therapy for Spasticity

ABSTRACT

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Spasticity is a condition resulting from excess motor neuron excitation, leading to involuntary muscle contraction in response to increased velocity of movement, for which there is currently no cure. Existing symptomatic therapies face a variety of limitations. The extent of relief that can be delivered by ablative techniques such as rhizotomy is limited by the potential for sensory denervation. Pharmacological approaches, including intrathecal baclofen, can be undermined by tolerance. One potential new approach to the treatment of spasticity is the control of neuromuscular overactivity through the delivery of genes capable of inducing synaptic inhibition. A variety of experiments in cell culture and animal models have demonstrated the ability of neural gene transfer to inhibit neuronal activity and suppress transmission. Similarly, enthusiasm for the application of gene therapy to neurodegenerative diseases of motor neurons has led to the development of a variety of strategies for motor neuron gene delivery. In this review, we discuss the limitations of existing spasticity therapies, the feasibility of motor neuron inhibition as a gene-based treatment for spasticity, potential inhibitory transgene candidates, strategies for control of transgene expression, and applicable motor neuron gene targeting strategies. Finally, we discuss future directions and the potential for gene-based motor neuron inhibition in therapeutic clinical trials to serve as an effective treatment modality for spasticity, either in conjunction with or as a replacement for presently available therapies.

Key Words: Spasticity, Transgene Expression, Motor Neuron, Gene Targeting

Spasticity is a condition resulting from excess motor neuron excitation caused by lesions in the upper motor neuron pathway that lead to the absence of inhibition of alpha and/or gamma motor neurons. This loss of inhibition results in involuntary muscle contraction, causing stiffness interfering with movement, speech, and locomotion.^{1,2} Affecting more than 12 million people worldwide, spasticity is commonly caused by stroke, multiple sclerosis, cerebral palsy, cerebral infection (encephalitis/meningitis), and/or cerebrospinal trauma.³ The most common clinical manifestations of spasticity are hypertonicity, clonus, muscle spasm, and/or fixed joints.^{4–7} Symptoms can present either intermittently (triggered by pain or other stimuli) or continuously, and they occur most commonly in response to increased velocity of movement.⁸

MECHANISM OF SPASTICITY

The exact mechanism of spasticity in humans is incompletely understood, primarily because it is multifactorial in nature. It is generally understood that spasticity is caused by pathology involving the stretch reflex, which normally causes a muscle to contract to resist the force that is stretching it. For normal movement to occur, the brain must be able to selectively turn this reflex off, usually via inhibitory signals relayed to the spinal cord via the corticospinal tract. However, damage to this circuit results in disinhibition of the stretch reflex; over time, this reduces the triggering threshold until excessive and complete muscle contraction can occur even at rest, making the limb virtually impossible to move.

Specific causes proposed include (a) alpha motor neuron hyperexcitability resulting from an imbalance in excitatory *vs.* inhibitory alpha motor neurons, and (b) gamma motor neuron hyperactivity manifesting as increased sensitivity of muscle spindle to stretch (fusimotor hyperactivity).⁹ Additional causes involve damage to descending tracts that control interneurons responsible for (a) mediating presynaptic inhibition of the Ia terminals on the alpha motor neuron, (b) mediating type II afferents, and (c) reciprocal Ia inhibition. Such damage results in greater afferent stimulus to the alpha motor neuron as a result of stretch, decreased inhibition from type II afferents, and loss of normal inhibition of antagonist muscle during muscle stretch. Finally, a mechanism of decreased recurrent inhibition from Renshaw cells as a consequence of supraspinal damage has also been proposed. Whereas each individual cause contributes to the clinical picture observed in spasticity, it is unlikely that any single hypothesis is sufficient to explain the exact mechanism of spasticity.

EXISTING THERAPIES AND LIMITATIONS

Medical Therapies

Although there are a number of oral medications available to treat spasticity, almost none are effective without significant side effects. The most common medications are diazepam, baclofen, and progabide, all of which are designed to increase presynaptic inhibition of alpha motor neurons by activation of γ -aminobutyric acid (GABA) receptors. However, these treatments are associated with a high incidence of adverse effects, including sedation, weakness, fever without infection, and elevated liver enzymes.^{10,11} Agents affecting ion flux in skeletal muscle, such as dantrolene, lamotrigine, and riluzole, share side effects of muscle weakness, sedation, and idiosyncratic hepatitis.^{11,12} Additional agents that act on monoamines, such as tizanidine, are associated

with a similar side-effect profile.^{11,13} These significant toxicities limit the doses of medication that can be employed, thereby limiting efficacy. Furthermore, the issue of tolerance significantly hinders the long-term efficacy of any pharmacologic therapy, particularly for baclofen and diazepam.

An alternative medical therapy involves the use of clostridial toxin (i.e., botulinum toxin), which acts by decreasing acetylcholine release at the neuromuscular junction, resulting in a neuromuscular blocking effect. However, the results from this therapy are often transient, with redosing complicated by tachyphylaxis, and increasing dosage complicated by severe muscle weakness.^{14–16} The issue of cost is another consideration. The cost of clostridial toxin treatment might hinder its extensive clinical applications because conventional oral therapies are much less expensive.¹⁷

Surgical Therapies

Surgery for spasticity is reserved for cases refractory to medical management or for those that cannot be medically managed because of intolerable side effects. The most common surgical options are generally orthopedic (consisting primarily of tendon-release operations) and neurosurgical.^{18,19} Neurosurgical procedures fall into two categories: nonablative and ablative. The most frequently used nonablative procedure is intrathecal baclofen (ITB), which is generally offered for refractory patients with chronic spasticity (>12 mos). To be considered a candidate, a patient must demonstrate a positive response to ITB at a test dose of less than 100 μ g, compared with no response to placebo.²⁰ Although highly effective in improving muscle tone and reducing postoperative spasticity, ITB is fraught with catheter- and wound-related morbidity, both at the time of implantation and throughout the life of the implanted device.^{20,21}

There are limited data characterizing the problem of tolerance to ITB.^{22–25} In many cases, ITB tolerance is ascribed to progression of the underlying disease (in amyotrophic lateral sclerosis and multiple sclerosis) or to dynamic catheter obstruction (kinking), which is difficult to demonstrate on standard pump contrast injections (pumpograms) or nuclear medicine studies.^{23,26} In our practice, we use inpatient externalized catheter ITB trials to address the question of baclofen tolerance. In this context, it is easy to assess the patency of the catheter and document the threshold for response to an intrathecal drug. Outpatient trials of bolus intrathecal injection can be attempted, but these are often misleading because of the inherent differences in the pharmacokinetics of bolus and pump injection. However, the majority of our patients require gradually escalated doses of ITB to maintain adequate control of spasticity.

Another nonablative option is spinal cord stimulation, which has been shown to facilitate spasticity control, spasm inhibition, and gait improvement in spastic patients,²⁷⁻²⁹ likely by selective modification of segmental spinal reflexes.³⁰ However, efficacy varies greatly, is highly dependent on both electrode position and the degree of stimulation, and has questionable cost-effectiveness.^{31,32} Furthermore, spinal cord stimulation is fraught with device-related morbidity related to infection, electrode migration, wire breakage at the connector site, skin breakdown over the lead, and development of high impedance.³³

The most common ablative procedure is selective dorsal rhizotomy, which uses intraoperative electromyography and stimulation to identify the rootlets most responsible for causing severe spasticity.^{34,35} Selective dorsal rhizotomy has consistently been proven to reduce spasticity of cerebral palsy in children, with earlier age at surgery associated with a reduced incidence of lower-extremity deformities (i.e., contractures, secondary skeletal torsion) requiring orthopedic surgery later in life.³⁶⁻⁴⁰ However, selective dorsal rhizotomy comes with morbidity as well, related both to the risk of surgery and the reported increased incidence of hip subluxation after treatment.⁴¹ Furthermore, selective dorsal rhizotomy has not been shown to impact alpha motor neuron-induced spasticity.^{34,35} Less common ablative procedures include percutaneous radiofrequency rhizotomy and surgical myelotomy (dorsal root entry zone procedures), each of which provides moderate efficacy in exchange for a permanent central nervous system (CNS) lesion and possible supplementation with subsequent ablative procedures.⁴²⁻⁴⁵

POSSIBLE FUTURE THERAPIES

Despite the wide range of medical and surgical treatments for spasticity, there is currently no treatment modality that is hardware free, reversible, adjustable, nondestructive, and not subject to tolerance.^{2,3,14-16,46} A potential modality for satisfying these criteria is the use of viral vectors to elicit effects on muscle contraction via gene transfer, because selective control of certain genes has been shown to modulate neuronal activity in multiple applications.⁴⁷⁻⁵² Typically, motor neurons communicate with muscle fibers by releasing acetylcholine at the neuromuscular junction. The release of acetylcholine causes an excitatory postsynaptic potential in the muscle fiber that triggers a postsynaptic action potential, which then causes the muscle fiber to contract. Because spasticity results from excess excitation of motor neurons, transgene-induced inhibition of motor neuron excitatory responses could alleviate or even abolish the clinical manifestations of spasticity while evading the problems associated with tolerance. It is critical to achieve motor neuron-specific gene

transfer to achieve targeted therapeutic effects in spasticity treatment. With the development of vector targeting technology, a specific cell population, such as motor neurons, can be transduced selectively. This issue will be discussed in detail below.

POTENTIAL INHIBITORY TRANSGENES

One of the most widely studied inhibitory transgenes is the gene encoding glutamate decarboxylase (GAD), the rate-limiting enzyme required to produce the inhibitory neurotransmitter GABA. In vitro and in vivo studies using retroviral vectors and adeno-associated virus (AAV) vectors have suggested that it is feasible to achieve long-term GAD expression in the CNS.⁵³⁻⁵⁶ Studies also have shown that GAD expression in the CNS induces GABA production in vector-transduced cells. Transfer of GAD using viral vectors therefore bears the potential application in disorders resulting from overexcitation in the nervous system. In a rat Parkinson disease model, researchers have transferred GAD genes in an AAV vector into the glutamatergic neurons of the subthalamic nucleus.⁵⁷ GAD was expressed, and the expression of GAD induced production of GABA in these neurons. These findings suggest that AAV-mediated GAD gene transfer might provide a treatment option for overactive diseases such as Parkinson disease.^{57,58} These results have led to an ongoing Phase I trial of GAD gene transfer to the human subthalamic nucleus for medically refractory Parkinson disease patients.⁵⁸ In addition to GAD, two other transgenes have emerged as candidates that are potentially capable of providing the inhibitory impact on motor neurons necessary for treatment of spasticity. The performance of these transgenes in previous work has generated optimism for their potential in combating spasticity.

Tetanus Toxin Light Chain

Our laboratory has focused on the gene for the light chain (LC) fragment of clostridial neurotoxin. The expression of this gene in neurons provides inhibition of synaptic function in transgenic mice via reversible suppression of glutamatergic neurotransmission.^{59,60} Clostridial intoxication in neurons involves the production of inactive single-chain clostridial neurotoxin polypeptides, which are released after bacterial lysis. This release converts the polypeptide from an inactive single-chain molecule to an active di-chain molecule composed of a heavy chain (HC) and an LC fragment linked by a single disulfide bond. HC binds axon terminals and triggers internalization of the toxin. Once inside the neuron, reduction of the disulfide bond releases the active LC fragment. The activated LC cleaves the soluble N-ethylmaleimide-sensitive factor attachment receptor proteins responsible for synaptic vesicle membrane fusion.⁶¹⁻⁶³ Reduction in vesicle fusion inhibits neurotransmission without inducing neuronal cell death.^{59,61,62} Recent in vitro

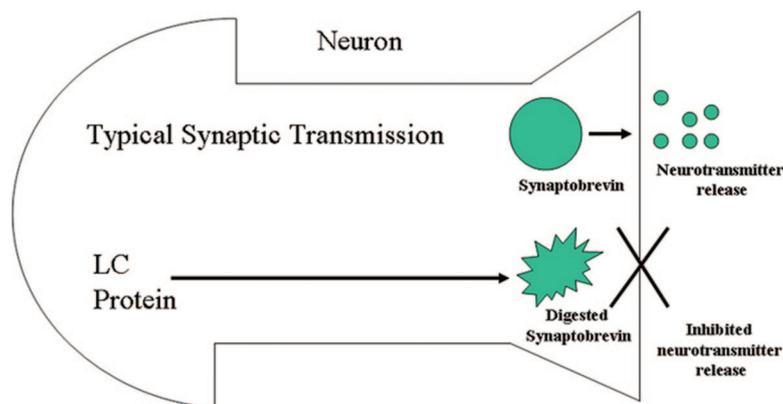


FIGURE 1 Depiction of typical synaptic transmission in a motor neuron, followed by the impact of tetanus toxin light chain (LC) on neurotransmitter release.

work in our laboratory has demonstrated that biologically active clostridial LC proteins can be successfully produced in cultured cells through viral gene transfer. The in vitro expressed LC protein was able to digest synaptobrevin, a soluble N-ethylmaleimide-sensitive factor attachment receptor protein that is involved in neurotransmitter release. Our in vivo experiments demonstrated that injection of an LC-expressing adenoviral vector into the rat spinal cord or the dSC nucleus in the brain stem inhibited neurotransmitter release (Fig. 1). The surrounding CNS structures were not found to be affected by LC gene expression during the 1-mo observation period.⁵⁹ Such specific, reversible, effective neuronal inhibition makes LC a viable transgene candidate for exploring gene-based treatment of spasticity.

Inwardly Rectifying Potassium Channel Kir2.1

Another gene of interest as a feasible modulator of motor neuron activity is Kir2.1, which encodes inwardly rectifying potassium (Kir) channels in the heart and brain. For inwardly rectifying potassium channels, the inward flow of potassium ions at subthreshold is greater than the outward flow of potassium ions for the opposite driving force. This inward rectification results when intracellular magnesium ions and polyamines enter the ion channel pore from the cytoplasmic side but are unable to pass through it to the extracellular solution. The block is more intense at decreased membrane potentials as the larger depolarization facilitates the movement of magnesium ions and polyamines into the pore. In contrast, as membrane potentials approach the resting membrane potential, the decreasing depolarization hinders magnesium ion movement into the pore. When the membrane potential exceeds the resting membrane potential (hyperpolarization), magnesium ions become prevented from entering the channel. Inwardly rectifying potassium channels prevent the

membrane potential from depolarizing by increasing the membrane potassium conductance. This increase in potassium permeability counterbalances the excitatory synaptic potentials that drive the initial membrane depolarization, hence inhibiting the formation of the action potential. In this way, the Kir2.1 contributes to stabilizing the resting potential at a sufficiently negative level to prevent enough sodium channel availability for action potential in the CNS and heart. Kir2.1 has been demonstrated to inhibit both evoked and spontaneous activity of neurons in vitro.⁶⁴

Thus, overexpression of inwardly rectifying potassium channels in motor neurons has the potential to inhibit depolarization, resulting in a subsequent drop in action potential generation and inhibition of neuromuscular transmission (Fig. 2). Previous work has demonstrated that Kir2.1 can be successfully transferred into cultured neurons, resulting in selective, inducible, reversible genetic expression of neuronal excitability.⁶⁴ For these reasons, the gene for Kir2.1 and other inwardly rectifying channels may provide a means for the control of motor neuron overactivity, thus providing an approach to spasticity.

STRATEGIES FOR CONTROL OF TRANSGENE EXPRESSION

Inducible Gene Expression System

Gene therapy will only prove beneficial as a treatment modality for spasticity if it provides advantages over existing pharmacologic and lesion-based modalities. For this to be accomplished, transgene expression must be both adjustable and reversible. One way to control viral vector-mediated transgene expression is to use inducible promoter elements. Several inducible promoter systems have been developed for this purpose, such as tetracycline, RU-486, rapamycin responsive systems, and the chimeric drosophila/bombyx ecdysone receptor system.⁶⁵⁻⁶⁸ As an illustration of how

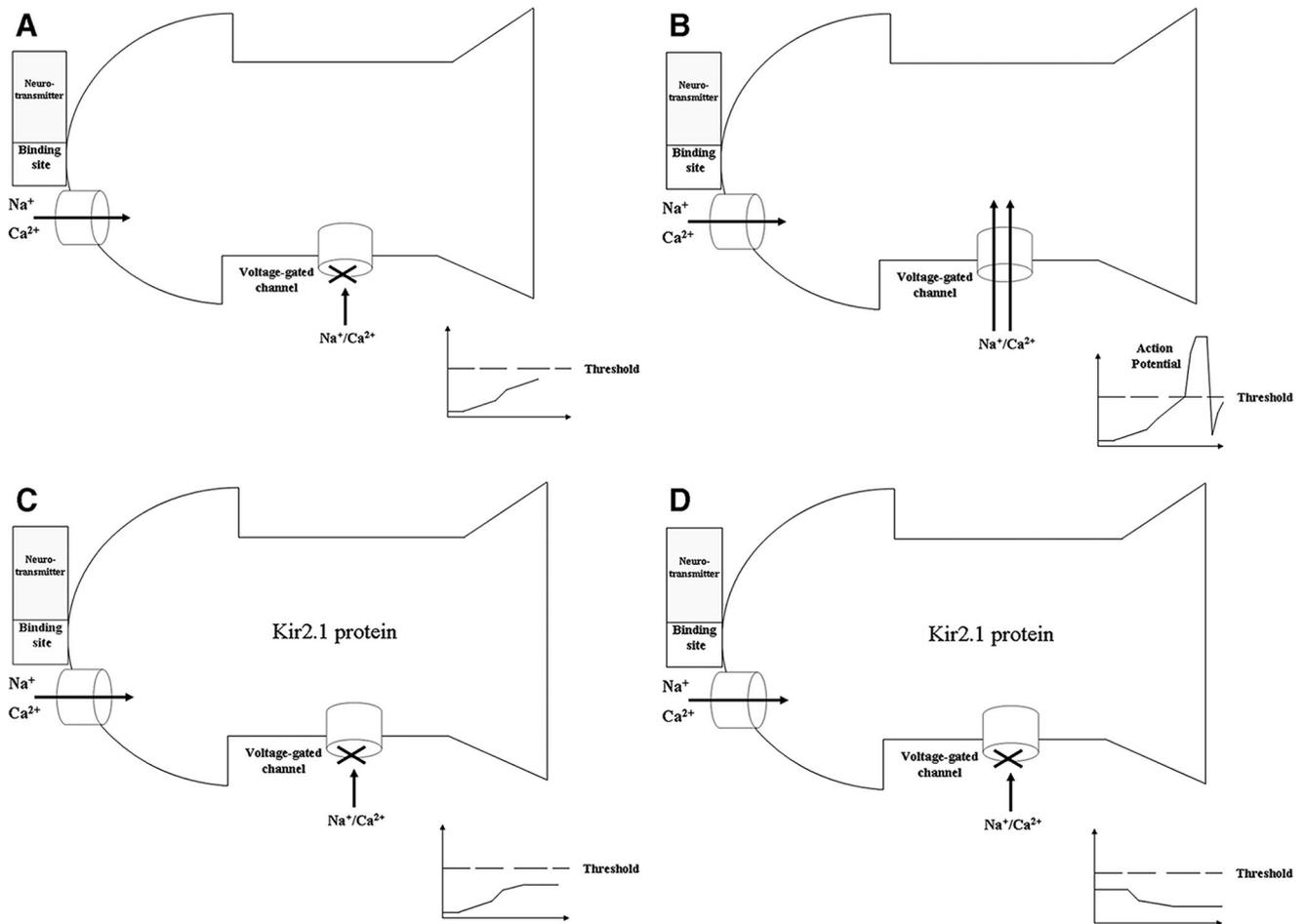


FIGURE 2 Mechanism of action of Kir2.1 in motor neurons. A, Typical motor neuron with voltage at resting potential. The neurotransmitter binds, resulting in an influx of cations (sodium and calcium), driving the voltage toward the threshold as depolarization occurs. B, The excitatory postsynaptic potential reaches its threshold and induces opening of voltage-gated channels, resulting in a large influx of sodium ions, triggering the action potential. C, Motor neuron at resting potential with Kir2.1 introduced. Kir2.1 overexpression clamps the resting membrane potential to the reversal potential of K⁺ ions and makes it more resistant (relative to A) to depolarizing forces caused by neurotransmitter binding. D, The excitatory postsynaptic potential displaces the magnesium ion (Mg²⁺) as Kir drives repolarization. The Kir-driven efflux of K⁺ balances the influx of Na⁺, thereby preventing the voltage from reaching its threshold, and keeping the voltage-gated channels closed. This inhibition allows for control of neuromuscular transmission.

inducible systems work, we will discuss the tetracycline responsive promoter system, of which there are two types. The first can be turned on in the presence of doxycycline (tet-on), and the second can be turned off in the presence of doxycycline (tet-off). In the tet-on system, the transgene is under the control of cytomegalovirus immediate early (CMVie) promoter, which is composed of a cytomegalovirus (CMV) minimal promoter and seven repeat sequence from a bacterial tet repressor DNA binding sequence (tetO). The other component of the tet-on system is a cassette that expresses tetracycline responsive transactivator proteins. In the presence of doxycycline, the complex formed between tetracycline responsive transactivator protein and doxycycline binds to CMVie promoter to turn on downstream therapeutic transgene expression.

As alluded to above (and true for other inducible systems), two components are required to achieve regulated gene expression. Because some of the compounds used to induce gene expression for these systems can pass the blood–brain barrier, they can be applied to the control of gene expression in the CNS. As such, they hold promise as a means to control transgenes expressed in the spinal cord broadly and motor neurons specifically, rendering them viable for the treatment of spasticity. Systemic delivery and intrathecal infusion of these trigger compounds have been proposed as means to control antispasticity transgenes. Because the candidate genes to treat spasticity encode proteins that either shut down neurotransmission (LC) or induce hyperpolarization (Kir), an inducible system could potentially limit undesirable side effects of gene expression. Furthermore, the use of an inducible expression system may

provide a way to adjust gene expression levels according to symptom severity.

MOTOR NEURON TARGETING STRATEGIES

A variety of strategies exist for targeting gene delivery to specific cell populations. Vectors can be designed to specifically bind to neurons in general or to motor neurons specifically. Further, cell type-specific promoters can be used to restrict gene expression to defined neuronal populations. As discussed earlier, existing pharmacologic therapies for spasticity (including ITB) have been limited by off-target effects that create side effects at higher doses. These off-target effects result from the binding of these agents to neurons in a variety of functional systems other than the desired motor neuron and spasticity-inducing reflex. The ideal scenario, therefore, is the use of the most minimally invasive approach that would permit the introduction of an efficient, controllable gene into the neurons controlling the spasticity-inducing reflex. Spinal cord motor neurons are the primary targets for gene delivery in the treatment of spasticity. Both promoter-level motor neuron targeting, and enhanced motor neuron binding and uptake through vector capsid modifications, are currently being pursued.

Direct Injection

Animal experiments have demonstrated that direct spinal cord injection of viral vectors is a feasible, albeit risky, way to introduce genetic material into the spinal cord. Furthermore, our laboratory and others have reported gene transfer to motor neurons via direct injection of a wide variety of viral vectors into the animal spinal cord. However, diffuse expression of transgenes that cause neural inhibition would be predicted to affect sensory systems as well as motor systems. Such a diffuse effect would be acceptable in the context of the treatment of spastic paraplegia, but not for patients with preserved sensory and motor function. Another concern related to direct spinal cord injection is the potential disinhibition of motor neurons attributable to inhibitory gene expression in spinal cord interneurons. As such, effective motor neuron targeting is critical for the treatment of spasticity using gene transfer. Currently, two strategies exist to achieve motor neuron-specific gene transfer. The first strategy is to genetically modify viral vector surface proteins with a short motor neuron-specific peptide to render these vectors motor neuron specific.⁶⁹ The second strategy is the application of motor neuron-specific promoters to confine gene expression to motor neurons.⁷⁰

A variety of tissue-specific and cell-specific promoters have been tested for gene transfer. Myelin

basic protein is expressed in oligodendritic cells and Schwann cells in vivo. Previous studies have shown that myelin basic protein-directed oligodendrocyte-specific green fluorescent protein gene expression occurs mainly in the white matter but not in other cell types such as neurons, astrocytes, or microglial cells.^{71,72} Other tissue-specific promoters including neuro-specific enolase promoter,²³ platelet-derived growth factor β -chain promoter, or β -glucuronidase promoter have also been studied for tissue-specific gene expression.⁷³⁻⁷⁵

Although similar promoter-level restriction of gene expression has yet to be achieved for motor neurons, the study of cell differentiation has led to the recognition of several genes that are specifically expressed in neural progenitors as they mature into motor neurons. The identification of the promoters that control the expression of these genes provides a tool to achieve motor neuron-specific therapeutic gene expression. HB9, a homeodomain transcription factor, is expressed selectively by postmitotic spinal motor neurons in the developing vertebrate CNS and serves as a marker for the motor neuron phenotype.⁷⁶ A 9-kb *Hb9* promoter sequence from the 5' *Hb9* gene has been shown to direct motor neuron-specific gene expression in in vivo studies.⁷⁷ Furthermore, Nakano et al.⁷⁰ have isolated a 125-bp enhancer sequence from the homeobox gene promoter (HB9) region that restricted gene expression to the spinal cord motor neurons in transgenic animals. This enhancer/promoter sequence will be particularly useful because it is small enough to be accommodated in almost all available gene-transfer vectors. Interestingly, Pramatarova et al.⁷⁸ have reported that neurofilament LC promoter directed spinal cord motor neuron-specific gene expression of superoxide dismutase 1 mutation G37R. We anticipate that this same promoter could be used to drive motor neuron-specific gene expression for the treatment of spasticity in the future. Finally, the promoters for the genes that control acetyl choline production and metabolism can be leveraged to design motor neuron-specific expression systems.

Although promising because of its ability to achieve high levels of gene expression, the delivery method of direct injection carries with it two major problems. The first is that it requires stable gene expression for a long period of time because repeated invasive surgery is not desirable. Secondly, the possibility of spinal cord trauma from direct spinal cord injection represents a serious source of morbidity. For these reasons, design of vectors capable of enhanced retrograde axonal transport (remote delivery) has been investigated as an alternative to the direct injection strategy.

Remote Injection

Remote delivery of therapeutic vectors can bypass direct CNS trauma by vector injection into innervated muscle groups and peripheral nerves. This delivery method has the advantage of facilitating repeated application with relatively low risk to the patient. Once injected, the foreign genetic material can be ferried into lower motor neurons of the spinal cord via retrograde axonal transport.^{79,80}

Skeletal muscles are innervated by nerve fibers from lower motor neurons in the spinal cord or brain stem. Active material transport between the nerve terminals and the cell body in both anterograde and retrograde directions exists to support the metabolic needs of these remote terminals. Therefore, it is theoretically possible to inject appropriately designed therapeutic agents into the muscle and to have the injected agents transported back to the cell body through retrograde axonal transport. A variety of viruses and toxins have evolved to capitalize on this conduit into the CNS. For example, the rabies virus can be transported to CNS neurons after peripheral inoculation, and the herpes virus can be transported into ganglion neurons from peripheral inoculation sites.⁸¹ Clostridial tetanus toxin also can be retrogradely transported to the CNS neurons from peripheral inoculation sites. Not surprisingly, these properties all have been tested in gene-transfer vector design to achieve central targeting from peripheral inoculation.

Axon terminal uptake of the rabies virus at the neuromuscular junction depends on the rabies G glycoprotein.⁸² Consequently, efforts have been made to pseudotype or coat gene-transfer vectors with this molecule. Mitrophanous et al.⁸³ have reported the development of an equine infectious anemia virus–based lentiviral vector pseudotyped with rabies G proteins for neuron-specific transduction. These vectors have been demonstrated to undergo avid uptake in innervated muscle fibers and enhanced retrograde transport into the related motor neurons in a retrograde fashion.

As mentioned above, tetanus toxin is composed of an HC and an LC and undergoes retrograde axonal transport to CNS neurons from peripheral inoculation. The HC component binds to its receptor GT1b and mediates the retrograde migration of the holotoxin. This property has been used to ferry therapeutic or tracer agents to CNS neurons, as the HC component has been used to deliver therapeutic agents such as superoxide dismutase I and cardiotropin-1 into motor neurons in attempts to target motor neuron diseases.^{84–87}

Despite these promising results, with currently available vector systems, only a low degree of gene-transfer efficiency can be achieved. Because a large amount of vectors are needed to generate a

favorable clinical benefit, this delivery method may increase the antigenic burden to the patients in addition to posing a manufacturing challenge. Additionally, because retrograde transport impacts the dorsal root ganglion as well as the desired motor neuron, potentially unwanted side effects may occur, as with the current modality of clostridial toxin therapy previously discussed.

With these limitations in mind, a discussion of gene therapy remains important for the treatment of spasticity because (a) gene delivery can control synaptic function, b) this control can be specifically targeted to motor neurons, and c) the expression of these genes can be controlled once applied to motor neurons. The development of a modality that is rapidly progressing to conquer these frontiers will, in the near future, mark a dramatic advance in the care that physicians can provide to patients with spasticity.

Viral Gene-Transfer Vectors

As alluded to above, gene-transfer vectors currently used in preclinical and clinical studies are mainly viral vectors. It is therefore appropriate to describe briefly the characteristics of some major viral vectors. Retroviral vectors, one of the earliest types of viral vectors developed for gene transfer, are still widely used in preclinical and clinical studies. Recombinant retroviral vectors can generally accommodate up to 8 kb of transgene. This group of viral vectors only infects dividing cells. Theoretically, they mediate long-term gene expression as the transgene is integrated into the host-cell genome. For the same reason, retroviral vectors may be tumorigenic because the transgene sequence and the viral long terminal repeat sequences may integrate randomly and activate a tumor-suppressor gene.⁸⁸ Adenoviral vectors are another group of vectors that are being used for gene transfer. Conventional adenoviral vectors can take about 7 kb of transgene sequence. The newer, gutless adenoviral vectors can accommodate up to 35 kb of foreign DNA sequence. Gene expression from adenoviral vectors is usually transient. The disadvantage of adenoviral vectors is that these vectors have the potential to trigger deadly immune responses.⁸⁹ AAV also can be genetically modified as a gene-transfer vector. AAV vectors can hold up to 5 kb of foreign DNA sequence. Gene expression from AAV vectors is usually long-lasting relative to adenoviral vectors. Although wild-type AAV integrates into a specific site on chromosome 19, recombinant AAV might lose this ability; therefore, it is not considered tumorigenic. The downside of AAV vectors is their relatively small packaging capacity of 5 kb, as mentioned above. Newer techniques, however, exist to increase their packaging capacity to more than 5 kb.⁹⁰ So far, AAV vectors have a good safety

profile in preclinical and clinical studies. There are also other gene-transfer vector systems such as herpes simplex virus, human immunodeficiency virus, and vaccinia virus-based vectors.^{91–93}

FUTURE DIRECTIONS

All of the transgenes discussed in the present review inhibit neuronal and synaptic activity in a relatively nonspecific fashion. As the mechanisms of spasticity are further elucidated, we anticipate that new candidate transgenes will emerge that will be capable of specifically blocking spasticity as opposed to general neuronal activity. The continued evolution of gene-transfer technology has provided the ability to transfer desired genetic material into human neurons. Practical motor neuron gene therapy for spasticity will depend on means for safe, durable, controllable, and specific gene delivery. Although an acceptable system that incorporates all of these features is not yet available, in the present review we have attempted to demonstrate progress in each of these dimensions. The continued dire need for motor neuron protection strategies for the treatment of amyotrophic lateral sclerosis and spinal muscular atrophy is driving the evolution of techniques for safe, durable, and specific motor neuron gene delivery. Similarly, the evolution of systems for controlled gene expression is being driven by the need for this feature in the therapy of a wide variety of diseases. The application of gene-based neural inhibition to the control of subthalamic nucleus overactivity in Parkinsonian humans presages the broader application of this approach to epilepsy, spasticity, pain, and other functional disorders of the nervous system. We anticipate that physicians will play an intimate role in the deployment of these therapies.

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