Biochemical and Biophysical Research Communications 388 (2009) 73–78

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effective neuropathic pain relief through sciatic nerve administration of GAD65-expressing rAAV2

Jaehyung Kim [a,b], Sung Jin Kim [c,d], Heuiran Lee [c,d,*,1], Jin Woo Chang [a,b,*,1]

[a] Department of Neurosurgery, Yonsei University College of Medicine, Seoul, Republic of Korea
[b] Brain Korea 21 Project for Medical Science & Brain Research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea
[c] Department of Microbiology, University of Ulsan College of Medicine, Seoul, Republic of Korea
[d] Research Institute for Biomacromolecules, University of Ulsan College of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 22 July 2009
Available online 28 July 2009

Keywords:
Neuropathic pain
Sciatic nerve
rAAV2
GAD65
GABA

ABSTRACT

Recently, we demonstrated that the administration of GAD65-expressing rAAV2 to DRG attenuates peripheral neuropathy by inducing GABA release in the spinal cord. However, the direct injection to DRG is invasive and may therefore cause nerve injury and other side effects.

To circumvent this surgical intervention, we explored the potential of a much simpler and less invasive route of sciatic nerve administration. Using a neuropathic pain model, we introduced rAAV2-GAD65 through sciatic nerve and examined its therapeutic potency in pain-related behavior tests. Both GFP and GAD65 expression indicated that effective transgene delivery to the DRG can be accomplished via sciatic nerve administration. Equally importantly, the GABA concentration in the spinal cord increased significantly after GAD65 introduction, and pain symptoms were dramatically reduced and persistently controlled. The implication is that the sciatic nerve is a highly promising route for delivering rAAV2 to the DRG, and thus represents a much less invasive, clinically viable gene therapy option.

Introduction

Neuropathic pain is a chronic pain syndrome that can be caused by various diseases, drugs, or damage to sensory neurons within the peripheral nervous system [1]. Peripheral neuropathic pain occurs as a result of aberrantly increased excitability of primary sensory afferents and central sensitization of nociceptive circuits in the ipsilateral dorsal horn [1–3]. There have been a number of recent advances in the development of therapeutic agents and techniques for treating chronic pain [4,5]. Nevertheless, nearly half of all neuropathic pain patients do not respond to currently available treatment options.

Encouraging results have come from animal studies, where substantial advances in establishing representative animal models for neuropathic pain have allowed novel therapeutic strategies to be validated. Animal studies employing various neuropathic pain models have provided solid evidence that gene-based therapy may be a promising alternative for patients. Gamma-aminobutyric acid (GABA), the product by glutamic acid decarboxylase (GAD), is a principal inhibitory neurotransmitter in the dorsal horn of the spinal cord and plays a crucial role in modulating synaptic circuits [6]. There are two isoforms of mammalian GAD encoded by two distinct genes [7]. GAD65 is present as a membrane-associated form in the synapses and is involved in producing a synaptic GABA for vesicular release [8,9]. Another isoform, GAD67, is generally distributed throughout the cell body and is primarily responsible for cytosolic GABA release through a non-vesicular mechanism [8–10]. GABA hypo-function, caused by the selective loss of GAD65-producing GABAergic neurons in the ipsilateral dorsal horn, has been well documented in various pain models [11,12]. Moreover, exogenous introduction of the GAD gene to the dorsal root ganglia (DRG) has been shown to increase GABA concentrations in the spinal dorsal horn, leading to attenuation of pain symptom [13].

Recombinant adeno-associated viral vector (rAAV), particularly serotype 2 (rAAV2), offers a number of advantages as a gene delivery vehicle, and has thus been widely utilized to treat central nervous system (CNS)-related chronic diseases [14–16]. rAAV2 is non-pathogenic, transduces post-mitotic neural cells, and provides long-term transgene expression. Recently, we showed that the delivery of GAD65 via rAAV2 enhanced GAD65 expression to the subthalamic nucleus and significantly reduced Parkinsonian symptoms in a rat model of the disease [17]. Using a neuropathic pain model, we also provided evidence that direct infection of rAAV2-GAD65 to DRG induces constitutive GABA production, resulting in persistent attenuation of pain symptoms [18].

* Corresponding authors. Address: Department of Microbiology, University of Ulsan College of Medicine, Songpa P.O. Box 145, Seoul, Republic of Korea. Fax: +82 2 3010 4259 (H. Lee).
E-mail address: heuiran@amc.seoul.kr (H. Lee).
[1] These two authors contributed equally to this work as corresponding authors.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.07.120

However, direct injection into DRG is invasive, requiring a surgical operation that could cause nerve injury and other side effects. To resolve this limitation, we attempted to express GAD65 in DRG by administrating rAAV2 via the much simpler and less invasive sciatic nerve route. After introducing rAAV2-GAD65 via the sciatic nerve in a rat pain model, we characterized the expression of the GAD65 transgene and examined its therapeutic potency. The results demonstrate that pain symptoms are sharply reduced following the increase in GAD65 and GABA expression, suggesting that the sciatic nerve is an effective route for delivering transgene to the DRG.

Materials and methods

rAAV-GAD65 preparation. rAAVs were constructed and produced using the AAV helper-free system obtained from Stratagene (Kirkland, WA, USA). The viruses were produced as previously described in detail [17]. The numbers of total and infectious virus particles were estimated using an ELISA kit (Progen Inc., Heidelberg, Germany) and immunocytochemistry for GAD65 (Chemicon, CA, USA), respectively.

Animal care, surgical procedures and behavioral test. All animal experiments were conducted according to the guidelines of the Ethical Committee of International Association for the Study of pain [19] and the Institution Animal Care and Use Committee of Yonsei University. Male Sprague–Dawley rats weighing 180 g were used to generate the neuropathic pain rat model. Under pentobarbital sodium solution (50 mg/kg), the left tibial and sural nerves were carefully and tightly ligated with suture silk and then completely transected [20]. Two weeks after the surgical operation, rAAV2-GAD65 (3 μl) was injected into the left sciatic nerve [21]. Behavioral testing was performed before and after rAAV2 injection [18,20,22].

Immuno-based analysis. Immunohistochemistry was performed as described previously [18]. For immunoblotting analysis, the dorsal quadrant of the L1 to L2 of the spinal cord was homogenized (Intronbio, Korea) and centrifuged for 10 min at 12,000 rpm. Protein concentrations in supernatants were measured using the BCA protein assay reagent kit (Pierce, Rockford, IL, USA). Supernatant proteins were separated by 12% SDS–PAGE and transferred to membranes. After blocking, membranes were incubated with primary anti-GAD65/67 (1:8000; Chemicon, Temecula, CA, USA) and anti-actin (1:10,000; Sigma, St. Louis, MS, USA) antibodies, followed by HRP-conjugated secondary antibodies (1:2000; Serotec, Oxford, UK). Immunoreactive proteins were detected using ECL system (Pierce, Rockford, IL, USA), and the intensity of each band was determined by densitometric analysis (TINA, Japan).

HPLC analysis. The amount of *in vivo* GABA release per dorsal horn of the spinal cord was determined by HPLC, following microdialysis. Microdialysis and HPLC analysis was performed as described previously [18].

Statistical analysis. Data are presented as mean ± SEM. Statistical analyses were performed using a Kruskal–Wallis one way analysis of variance, followed by a Mann–Whitney *U*-test to compare the behavioral data from each group. *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA).

Results

Effective gene transfer by administering rAAV2 to the DRG through the sciatic nerve

A neuropathic pain model (the TST model) was generated by tight ligation and transection of the tibial and sural nerves, leaving common peroneal nerves intact [20]. A previous study suggested that neuropathic pain consistently develops following TST surgery, based on von Frey and pinprick mechanical paintests conducted over 3 months. To test the sciatic nerve as a route for delivering transgenes to the DRG, we first injected a green fluorescence protein (GFP)-expressing rAAV2 reporter construct (rAAV2-GFP) into the left sciatic nerve of the pain model rats ($n = 3$) and monitored GFP expression following cryo-preparation of the ipsilateral L4/L5 DRG. GFP was first observed within 1 week after injection (data not shown) and was heavily expressed in both DRG neurons at 3 weeks, as evidenced by bright green fluorescence accumulation (Fig. 1). This data thus implies that rAAV2 can mediate efficient gene expression in the DRG after being administered into the sciatic nerve.

Attenuation of pain symptoms after rAAV2-GAD65 administration in a rat neuropathic pain model

To investigate the possible beneficial effects of injecting GAD65-expressing rAAV2 into the sciatic nerve, we tested pain-related mechanical behaviors under blinded conditions, performing pinprick and von Frey tests for mechanical hyperalgesia and allodynia, respectively. Two weeks after surgery when pain symptoms were obvious, rAAV2-GAD65 was introduced into the sciatic nerve ($n = 6$). Surgery groups tested with saline ($n = 3$) or rAAV2-GFP ($n = 3$) served as pain control groups, and a non-surgical group ($n = 3$) was included as a normal control (Fig. 2). Both tests showed that rAAV2-GAD65 treatment sharply alleviated pain symptom. Two weeks after surgery, pain control groups responded positively to 8 of 10 stimuli in the allodynia test, and no responses were recorded for the normal control group (Fig. 2B). rAAV2-GAD65 introduction sharply subdued pain symptoms within 1 week, reducing

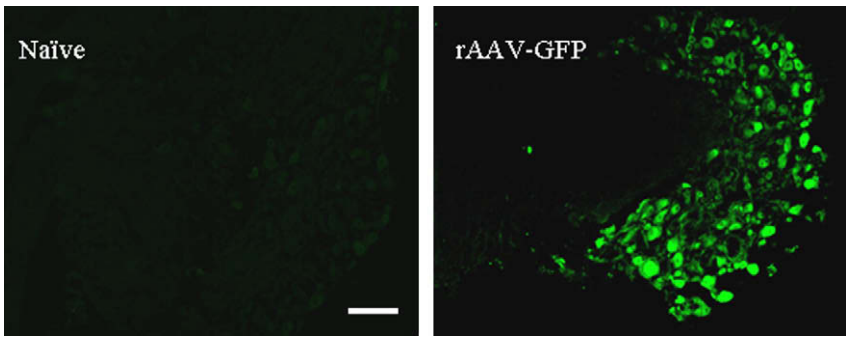

Fig. 1. rAAV2-mediated transgene expression in the DRG. After generating the pain model, 3 μl rAAV2-GFP (1.3×10^7 infectious particles/ml) was administrated through the sciatic nerve using a glass micropipette. Three weeks later, GFP expression in L4 and L5 ipsilateral DRG was determined by fluorescence microscopy following cryo-sectioning. GFP expressions were clearly detected in the rAAV2-GFP group, but not in the na group. (Original magnification: 200×; Scale bar: 50 μm).

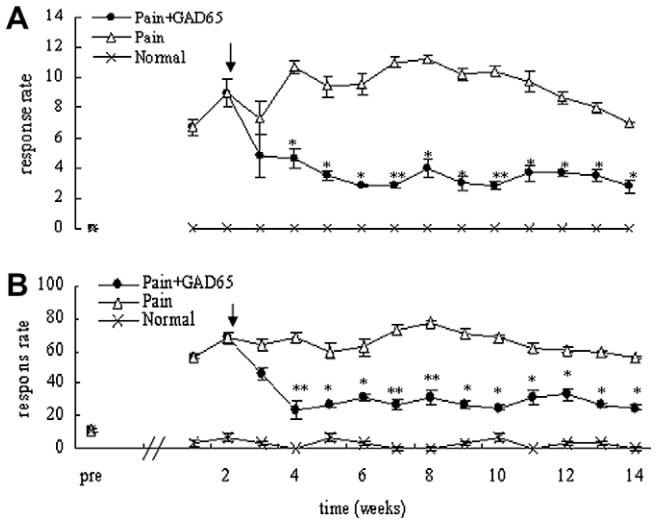

Fig. 2. Therapeutic effects of sciatic nerve-administered rAAV2-GAD65 determined by behavioral testing. Both mechanical hyperalgesia (A) and mechanical allodynia (B) were assessed following virus administration in the TST pain model. Rats were either unoperated (×), or administered rAAV2-GFP (△) or rAAV2-GAD65 (●) via the sciatic nerve 2 weeks after neuropathic surgery. Three microliters of rAAV2 (1.3×10^7 infectious particles/ml) was introduced. Data is expressed as mean ± SEM. Asterisks indicate significant differences between the rAAV2-GAD65 pain-test group and the rAAV2-GFP injected control group ($^{*}P < 0.05$, $^{**}P < 0.01$).

the number of positive responses to the allodynia test to 4.5 ± 0.3 responses ($P < 0.05$). Reduced allodynia persisted throughout the experimental period (>3 months). A similar pattern of improvement in hyperalgesia tests was evident in the GAD65-administered pain-test group (Fig. 2A). Behavior tests thus imply that pain symptoms can be effectively eased by administration of rAAV2-GAD65 through the sciatic nerve.

Increased GAD65 expression and GABA levels following rAAV2-GAD65 injection into the sciatic nerve

To examine whether GAD65 was exogenously expressed in ipsilateral DRG following rAAV2-GAD65 administration, we assessed GAD65 transgene expression in DRG neurons after completion of behavioral testing (14 weeks post-injection). A representative example of GAD65-specific immunostaining is shown in Fig. 3A. A quantitative analysis of immunostained images indicated that GAD65 expression was significantly increased after rAAV2-GAD65 administration compared to that in pain control groups (Fig. 3B). Western blot analysis of spinal cord extracts showed that GAD levels in pain control groups were significantly decreased (50.0% ± 0.2%) compared to those in the normal control group (Fig. 4A and B), a phenomena that has been well documented following pain surgery. In the rAAV2-GAD65-administered group, there was a consistent and significant increase in GAD, which recovered to levels up to 120.0% ± 0.2% of those in the normal group. HPLC analysis was also carried out to evaluate GABA levels in the spinal dorsal horn in the DRG. Fig. 4C shows that GABA levels increased dramatically in the rAAV2-GAD65-injected group, reaching a concentration of 0.523 ± 0.033 pmol compared to only 0.314 ± 0.065 pmol in the GFP-pain control group. Taken together, these results suggest that the introduction of rAAV2-GAD65 leads to a significant recovery in GAD65 expression in both ipsilateral DRG and the spinal cord which subsequently leads to a subsequent restoration of GABA levels in the dorsal horn.

Reduced c-fos activation in the rAAV2-GAD65-administered pain-test group

c-fos expression is an indirect biological marker of nociceptive processes [23]. In pain model, fos-like immunoreactivity in the ipsilateral lumbar segment of the spinal cord has been found to significantly increase [24]. An immunohistochemical examination showed

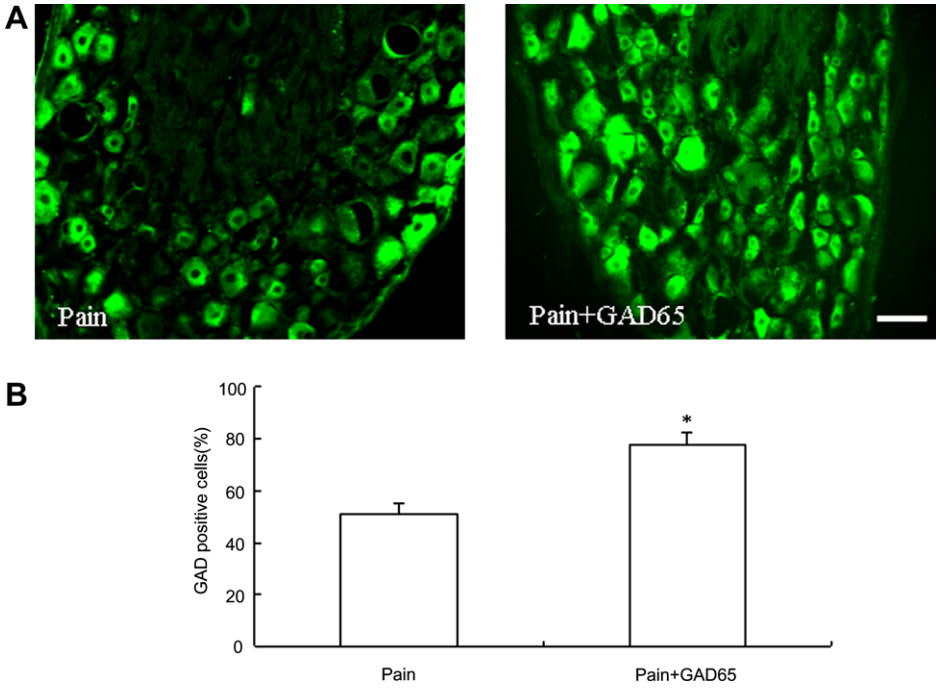

Fig. 3. Expression of GAD65 in the DRG. Immunohistochemical detection of GAD65 in the DRG after injection with rAAV2 vectors (A). Significant intensification of GAD65 expression was noticed in the DRG from pain model with rAAV2-GAD65 (B). Three microliters of rAAV2-GAD65 (6.2×10^7/ml; infectious particles) was injected to each L4, L5 DRG through the sciatic nerve injection ($^{*}P < 0.05$; Original magnification: 200×; Scale bar: 100 μm).

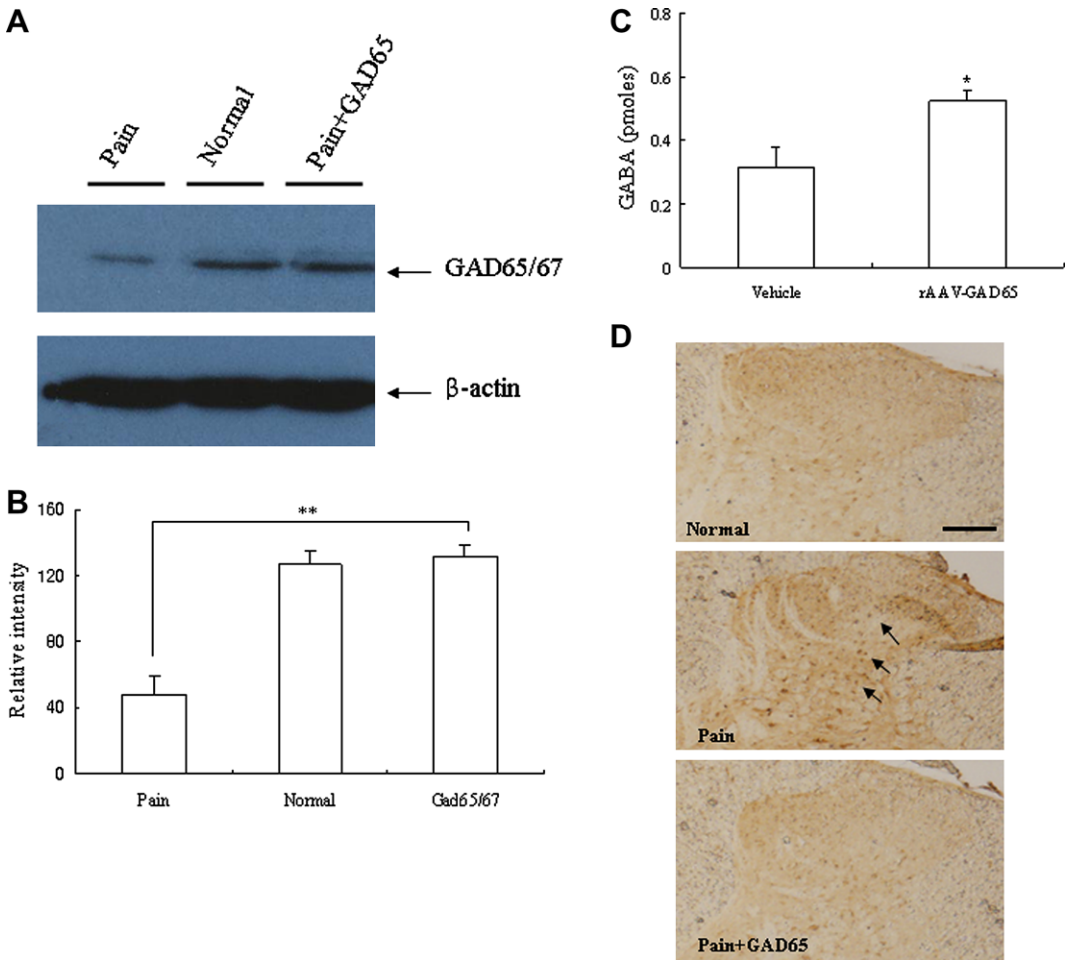

Fig. 4. GAD expression (4A,B), GABA secretion (C) and c-fos detection(D) in spinal dorsal horn. Animals were sacrificed immediately after completing the final behavioral tests; ipsilateral spinal dorsal horns were harvested and analyzed by Western blotting. (A) Representative Western blot. (B) Relative GAD expression. A substantial increase in GAD expression was observed in the rAAV2-GAD65-administered group. (C) GABA released from nerve terminals in the spinal dorsal horn was extracted *in vivo* from the CSF by microdialysis. The concentration of GABA in microdialysates was determined by HPLC 8 weeks post-surgery. GABA levels increased substantially in the rAAV2-GAD65-treated group in comparison to sham controls. (D) Immunohistochemical detection of c-fos-positive neurons in the ipsilateral lumbar segment after injection of rAAV2-GAD65. c-fos expression decreased significantly in the ipsilateral lumbar spinal cord from the rAAV2-GAD65 pain-test group ($^{*}P < 0.05$, $^{**}P < 0.01$; Original magnification: 200×; Scale bar: 100 μm).

substantial, unambiguous differences in the number of fos-positive cells among the pain control group, sham, and rAAV2-GAD65 injected pain group (Fig. 4D). Numerous c-fos-positive cells were observed in the pain control group, while only a few c-fos-positive cells were evident in GAD65-administered and normal groups. Additionally, there was no increase in c-fos expressing cells in the contralateral dorsal horn, regardless of experimental groups (data not shown).

Discussion

Neuropathic pain is a chronic neurodegenerative disease caused by diverse neural system dysfunctions caused by multiple etiological factors [1,2]. In the face of this heterogeneity, genetic therapy has emerged as a potentially more powerful approach to resolving pain symptoms than conventional pain-mitigating strategies. Here, we used a rat neuropathic pain model to show that (i) the therapeutically significant GAD65 gene can be effectively delivered to the DRG via the sciatic nerve as an rAAV2-GAD65 construct, (ii) GABA concentrations in the spinal cord significantly increase as a result of GAD65 delivery, (iii) pain symptoms are dramatically and persistently lessened concomitant with GABA increase, and (iv) c-fos activation, a biochemical nociceptive marker, sharply decreases in the GAD65-treated pain group.

A wide variety of vector systems, of both viral and non-viral origin, are currently available [25,26]. Among these, rAAV vector has been shown to very effectively transport therapeutic genes into the CNS and is considered one of the most appropriate tools for CNS-related gene therapy [27]. It also benefits from a set of extraordinary characteristics, including long-term gene expression, absence of vector-associated toxicity or substantial immune response, and physical stability of virus particles [27–30]. The clinical potential of rAAV is currently under investigation in human trials designed to validate the potency of this system against several neurodegenerative diseases, including Parkinson's disease [31]. Herpes simplex virus (HSV)-based vectors have also been explored in various pain models for their potential usefulness in delivering various therapeutic genes [24,32–35]. In these experiments, HSV is typically administrated to the subcutaneous footpad of experimental animals [36]. Therefore, HSV exhibits retrograde transport to the DRG within 1 week, and ultimately exerts effective therapeutic effects. One limitation of HSV-based treatment is the relatively short duration of the pain-killing effect, which requires repeated (i.e., at least every 2 months) HSV administration [13,24]. In contrast, rAAV-mediated gene expression is much more persistent: in our study, a single dose allowed consistent transgene expression, with no evidence of a decrease in transgene expression during

the experimental period [37–40]. Our study results are in good agreement with previous studies demonstrating the persistence of transgene expression by rAAV (over several months) in multiple CNS compartments.

In focused gene therapy, therapeutic genes are directly introduced into target cells, making gene products exclusively available within a specific target tissue in a regionally concentrated manner. Continuous focal expression of the transgene not only maximizes therapeutic effects, but also minimizes off-target effects. Multiple reports have described attempts to achieve local gene expression in the DRG/dorsal horn by various routes [17,40]. The most direct technique is administration of the vector into the DRG. This technique, however, requires invasive surgery that typically involves removing part of a spinal vertebra. Intrathecal administration is an alternative route that reduces dose requirements and off-target adverse events [14,39], but it is less effective than direct introduction. In contrast, sciatic nerve injection is an attractive strategy for local delivery of transgene to the DRG/spinal dorsal horn. Administration to the sciatic nerve is not as invasive as direct injection into the DRG, and is readily applicable to clinical situations [21]. Taken together with previous reports, our study also suggests that rAAV2-mediated gene delivery via the sciatic nerve effectively distributes the transgene to the DRG and the corresponding spinal cord. Thus, the sciatic nerve is an excellent route for local treatment of the DRG/spinal dorsal horn by an rAAV vector.

Even though neuropathic pain models have shown a selective loss of GAD65 expression, not GAD67-production, in GABAergic neurons of the ipsilateral dorsal horn [12,24], introduction of either GAD65 or GAD67 to the DRG has been implied to effectively relieve pain symptoms [13,24]. Both genes are capable of utilizing glutamic acid to generate GABA, a major inhibitory neurotransmitter in the spinal dorsal horn [9]. Therefore, these positive therapeutic outcomes may suggest that the recovery of GABA levels by either enzyme is sufficient to subdue pain symptoms caused by the loss of GAD65 neurons. However, because the experimental details, including gene delivery constructs, were quite differed in these reports, this conclusion remains tentative. Until these two isoforms are tested under identical condition, it remains possible that differences exist in the therapeutic potency (or other effects) associated with the administration of GAD65 or GAD67. Currently, Fink et al. are conducting a phase I trial with patients experiencing pain from terminal cancer using HSV-GAD67 [41]. Hopefully, this clinical trial will offer practical guidance for assessing the potential of GAD as a therapeutic gene.

In conclusion, this study demonstrates that the administration of rAAV2 containing GAD65 to the sciatic nerve greatly relieves neuropathic pain symptoms in a partially ligated/transected sciatic nerve rat model. Pain relief was accomplished within 1 week through increased levels of both GAD65 and GABA in the DRG and ipsilateral spinal dorsal horn, respectively. A single injection of rAAV2-GAD65 effectively attenuated pain symptoms for several months without any indication of loss in therapeutic efficacy. Thus, the present study provides clear evidence that the delivery of rAAV2-transgene constructs via the sciatic nerve holds promise as a much less invasive and clinically applicable gene therapy strategy for the management of neuropathic pain.

Acknowledgments

We thank Maria Khim for critical reading of the manuscript. This work was supported by grants to J.W. Chang from the Industrial Technology Development Programs of Ministry of Knowledge Economy, Republic of Korea (10031270) and to H. Lee from the Korea Science and Engineering Foundation (R13-2008-023-01002), Republic of Korea.

Reference

[1] D. Bridges, S.W. Thompson, A.S. Rice, Mechanisms of neuropathic pain, Br. J. Anaesthesiol. 87 (2001) 12–26.
[2] J.N. Campbell, R.A. Meyer, Mechanisms of neuropathic pain, Neuron 52 (2006) 77–92.
[3] M.D. Christensen, A.W. Everhart, J.T. Pickelman, C.E. Hulsebosch, Mechanical and thermal allodynia in chronic central pain following spinal cord injury, Pain 68 (1996) 97–107.
[4] N.B. Finnerup, S.H. Sindrup, T.S. Jensen, Chronic neuropathic pain: mechanisms, drug targets and measurement, Fundam. Clin. Pharmacol. 21 (2007) 129–136.
[5] M.J. Millan, Descending control of pain, Prog. Neurobiol. 66 (2002) 355–474.
[6] Stoyanova II, Gamma-aminobutyric acid immunostaining in trigeminal, nodose and spinal ganglia of the cat, Acta Histochem. 106 (2004) 309–314.
[7] M.G. Erlander, N.J. Tillakaratne, S. Feldblum, N. Patel, A.J. Tobin, Two genes encode distinct glutamate decarboxylases, Neuron 7 (1991) 91–100.
[8] S. Feldblum, A. Dumoulin, M. Anoal, F. Sandillon, A. Privat, Comparative distribution of GAD65 and GAD67 mRNAs and proteins in the rat spinal cord supports a differential regulation of these two glutamate decarboxylases in vivo, J. Neurosci. Res. 42 (1995) 742–757.
[9] J.J. Soghomonian, D.L. Martin, Two isoforms of glutamate decarboxylase: why?, Trends Pharmacol Sci. 19 (1998) 500–505.
[10] J. Mi, S. Chatterjee, K.K. Wong, C. Forbes, G. Lawless, A.J. Tobin, Recombinant adeno-associated virus (AAV) drives constitutive production of glutamate decarboxylase in neural cell lines, J. Neurosci. Res. 57 (1999) 137–148.
[11] J.M. Castro-Lopes, I. Tavares, A. Coimbra, GABA decreases in the spinal cord dorsal horn after peripheral neurectomy, Brain Res. 620 (1993) 287–291.
[12] K.A. Moore, T. Kohno, L.A. Karchewski, J. Scholz, H. Baba, C.J. Woolf, Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord, J. Neurosci. 22 (2002) 6724–6731.
[13] J. Liu, D. Wolfe, S. Hao, S. Huang, J.C. Glorioso, M. Mata, D.J. Fink, Peripherally delivered glutamic acid decarboxylase gene therapy for spinal cord injury pain, Mol. Ther. 10 (2004) 57–66.
[14] A.S. Beutler, M. Reinhardt, AAV for pain: steps towards clinical translation, Gene Ther. 16 (2009) 461–469.
[15] J.C. Grieger, R.J. Samulski, Adeno-associated virus as a gene therapy vector: vector development, Adv. Biochem. Eng. Biotechnol. 99 (2005) 119–145.
[16] D.M. McCarty, Self-complementary AAV vectors; advances and applications, Mol. Ther. 16 (2008) 1648–1656.
[17] B. Lee, H. Lee, Y.R. Nam, J.H. Oh, Y.H. Cho, J.W. Chang, Enhanced expression of glutamate decarboxylase 65 improves symptoms of rat parkinsonian models, Gene Ther. 12 (2005) 1215–1222.
[18] B. Lee, J. Kim, S.J. Kim, H. Lee, J.W. Chang, Constitutive GABA expression via a recombinant adeno-associated virus consistently attenuates neuropathic pain, Biochem. Biophys. Res. Commun. 357 (2007) 971–976.
[19] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110.
[20] B.H. Lee, R. Won, E.J. Baik, S.H. Lee, C.H. Moon, An animal model of neuropathic pain employing injury to the sciatic nerve branches, Neuroreport 11 (2000) 657–661.
[21] Y. Gu, Y. Xu, G.W. Li, L.Y. Huang, Remote nerve injection of mu opioid receptor adeno-associated viral vector increases antinociception of intrathecal morphine, J. Pain 6 (2005) 447–454.
[22] Q. Hogan, D. Sapunar, K. Modric-Jednacak, J.B. McCallum, Detection of neuropathic pain in a rat model of peripheral nerve injury, Anesthesiology 101 (2004) 476–487.
[23] G. Catheline, S. Le Guen, J.M. Besson, Intravenous morphine does not modify dorsal horn touch-evoked allodynia in the mononeuropathic rat: a Fos study, Pain 92 (2001) 389–398.
[24] S. Hao, M. Mata, D. Wolfe, S. Huang, J.C. Glorioso, D.J. Fink, Gene transfer of glutamic acid decarboxylase reduces neuropathic pain, Ann. Neurol. 57 (2005) 914–918.
[25] M.E. Martin, K.G. Rice, Peptide-guided gene delivery, AAPS J. 9 (2007) E18–E29.
[26] Y. Seow, M.J. Wood, Biological gene delivery vehicles: beyond viral vectors, Mol. Ther. 17 (2009) 767–777.
[27] L. Tenenbaum, A. Chtarto, E. Lehtonen, T. Velu, J. Brotchi, M. Levivier, Recombinant AAV-mediated gene delivery to the central nervous system, J. Gene Med. 6 (Suppl. 1) (2004) S212–S222.
[28] C. Burger, K. Nash, R.J. Mandel, Recombinant adeno-associated viral vectors in the nervous system, Hum. Gene Ther. 16 (2005) 781–791.
[29] Y. Lu, Recombinant adeno-associated virus as delivery vector for gene therapy – a review, Stem Cells Dev. 13 (2004) 133–145.
[30] P.E. Monahan, R.J. Samulski, AAV vectors: is clinical success on the horizon?, Gene Ther 7 (2000) 24–30.
[31] C. Mueller, T.R. Flotte, Clinical gene therapy using recombinant adeno-associated virus vectors, Gene Ther. 15 (2008) 858–863.
[32] J.R. Goss, W.F. Goins, D. Lacomis, M. Mata, J.C. Glorioso, D.J. Fink, Herpes simplex-mediated gene transfer of nerve growth factor protects against peripheral neuropathy in streptozotocin-induced diabetes in the mouse, Diabetes 51 (2002) 2227–2232.
[33] J.R. Goss, M. Mata, W.F. Goins, H.H. Wu, J.C. Glorioso, D.J. Fink, Antinociceptive effect of a genomic herpes simplex virus-based vector expressing human proenkephalin in rat dorsal root ganglion, Gene Ther. 8 (2001) 551–556.

[34] S. Hao, M. Mata, J.C. Glorioso, D.J. Fink, HSV-mediated expression of interleukin-4 in dorsal root ganglion neurons reduces neuropathic pain, Mol. Pain 2 (2006) 6.
[35] Z. Zhou, X. Peng, S. Hao, D.J. Fink, M. Mata, HSV-mediated transfer of interleukin-10 reduces inflammatory pain through modulation of membrane tumor necrosis factor alpha in spinal cord microglia, Gene Ther. 15 (2008) 183–190.
[36] M. Mata, J. Glorioso, D.J. Fink, Development of HSV-mediated gene transfer for the treatment of chronic pain, Exp. Neurol. 184 (Suppl. 1) (2003) S25–S29.
[37] B. Blits, M. Oudega, G.J. Boer, M. Bartlett Bunge, J. Verhaagen, Adeno-associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function, Neuroscience 118 (2003) 271–281.
[38] M.J. Eaton, B. Blits, M.J. Ruitenberg, J. Verhaagen, M. Oudega, Amelioration of chronic neuropathic pain after partial nerve injury by adeno-associated viral (AAV) vector-mediated over-expression of BDNF in the rat spinal cord, Gene Ther. 9 (2002) 1387–1395.
[39] B. Storek, N.M. Harder, M.S. Banck, C. Wang, D.M. McCarty, W.G. Janssen, J.H. Morrison, C.E. Walsh, A.S. Beutler, Intrathecal long-term gene expression by self-complementary adeno-associated virus type 1 suitable for chronic pain studies in rats, Mol. Pain 2 (2006) 4.
[40] Y. Xu, Y. Gu, P. Wu, G.W. Li, L.Y. Huang, Efficiencies of transgene expression in nociceptive neurons through different routes of delivery of adeno-associated viral vectors, Hum. Gene Ther. 14 (2003) 897–906.
[41] D. Wolfe, M. Mata, D.J. Fink, A human trial of HSV-mediated gene transfer for the treatment of chronic pain, Gene Ther. 16 (2009) 455–460.