American Pain Society
RESEARCH
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ADVOCACY
PUBLISHED BY
ELSEVIER

The Journal of Pain, Vol 16, No 3 (March), 2015: pp 283-290
Available online at www.jpain.org and www.sciencedirect.com

Injury-Specific Promoters Enhance Herpes Simplex Virus–Mediated Gene Therapy for Treating Neuropathic Pain in Rodents

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Abstract: Chronic neuropathic pain is often difficult to treat with current pain medications. Gene therapy is presently being explored as a therapeutic approach for the treatment of neuropathic and cancer pain. In this study, we sought to use an injury-specific promoter to deliver the mu-opioid receptor (MOR) transgene such that expression would occur during the injured state only in response to release of injury-specific galanin. To determine whether an injury-specific promoter can produce neuron-specific MOR expression and enhanced antinociception, we compared animals infected with a galanin promoter virus (galMOR) or a human cytomegalovirus promoter virus (cmvMOR). In behavioral assays, we found an earlier onset and a larger magnitude of antinociception in animals infected with galMOR compared with cmvMOR. Immunohistochemical analysis of dorsal root ganglion neurons revealed a significant increase in MOR-positive staining in cmvMOR- and galMOR-treated mice. Spinal cord sections from galMOR-treated mice showed a greater increase in density but not area of MOR-positive staining. These results suggest that using injury-specific promoters to drive gene expression in primary afferent neurons can influence the onset and magnitude of antinociception in a rodent model of neuropathic pain and can be used to upregulate MOR expression in populations of neurons that are potentially injury specific.

Perspective:* *An injury-specific promoter (galMOR) was used to drive MOR expression in a population- and injury-specific manner. GalMOR increased antinociception and density of MOR staining in the spinal cord. This article presents evidence that promoter selection is an important component in successful gene expression in an injury- and population-specific manner.

Key words: *Galanin, mu-opioid receptor, mechanical allodynia, thermal hyperalgesia, nerve injury.*

Chronic neuropathic pain often decreases quality of life, incurs high medical costs,[3,16] and is difficult to treat. The efficacy of current treatments varies across individuals and is associated with adverse side effects. Alternative treatments such as gene therapy are being explored in animal models of neuropathic pain[13-15,23,32] and in human cancer pain.[9,11] The use of recombinant herpes simplex virus type 1 (HSV-1) as a means to deliver genetic material to a targeted region in the peripheral nervous system is particularly beneficial because of its natural propensity for afferent neurons. Studies to date using HSV-1 as a gene therapy vector have produced changes in gene expression in the overall afferent neuron population with antinociceptive effects. The promoter influences the duration of changes in gene expression as well as the afferent population expressing the transgene. The cytomegalovirus (CMV) promoter is commonly used to drive gene expression as it is constitutively active and results in high levels of expression in a wide range of tissues.[25] Studies have explored the use of tissue-specific promoters in gene therapy to treat liver carcinomas,[10,34] cardiac ischemia,[29] and neurologic disorders.[4] However, using tissue- and

Received April 7, 2014; Revised December 13, 2014; Accepted December 13, 2014.
This work was supported by grants from the National Institutes of Health (R01 Grant NS26363), South Carolina Post Baccalaureate Research Education Program for Minorities (R25 Grants GM066526 and GM076277 from the National Institutes of Health), and Alfred P. Sloan Foundation.
The authors declare no conflicts of interest with respect to this study.
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1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2014.12.007

injury-specific targeting of transgenes to distinct afferent populations for the treatment of pain has not been explored. Galanin is normally present in low levels in dorsal root ganglia (DRGs) and the spinal cord. After injury that results in neuropathic pain–like behavior, galanin is highly upregulated proportionate to the extent of constriction injury and is used as a marker of nerve injury.[5,8,22,28] In the present study, we investigated the use of HSV-1 and an injury-specific galanin promoter to express mu-opioid receptors (MORs) specifically in injured primary afferent neurons. To determine whether an injury-specific promoter can enhance antinociception and drive expression of MOR in specific populations of afferent neurons as opposed to constitutive expression with a nonspecific promoter, we compared animals infected with the galanin-promoter virus (galMOR) to those infected with the CMV-promoter virus (cmvMOR).

Methods

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of South Carolina. Efforts were made to reduce the number of animals used and to minimize discomfort. Female Swiss Webster mice (5–6 weeks old, 20–25 g; Harlan Sprague Dawley Inc, Indianapolis, IN) were group-housed on a 12-hour light/dark cycle, with food and water available ad libitum.

L5 Transection

For L5 transection surgeries, animals were anesthetized with isoflurane, and all surgical procedures were performed under sterile conditions as previously described.[20] An incision over L5-S1 was made, the L6 transverse process was partially removed, and the L5 nerve was isolated and transected. In sham animals, an identical procedure was followed, but the L5 nerve was not transected. The fascia was closed with 3.0 polyester sutures. Seven days after L5 transection surgery, the mice were infected with virus as previously described.[31]

Viral Constructs and Infection

The following viral vectors have been described.[36] First, hsvCON, the control virus, expresses β-galactosidase (*Escherichia coli* lacZ), which is driven by the CMV immediate-early enhancer promoter. Second, cmvMOR (previously called SGZ) expresses MOR and is driven by the CMV immediate-early enhancer promoter (previously called SGMOR). Third, galMOR, a newly constructed virus, expresses MOR under the control of the galanin promoter (Fig 1). Animals were anesthetized with isoflurane, and 10 μL of the HSV-1 constructs (1×10^7 transduction units per microliter) were administered into the left plantar hind paw (n = 5–6/group).

Behavioral Testing

Animals were acclimated to the experimenter and testing environment prior to testing as previously described.[21] Mice were placed in acrylic glass enclosures over a mesh floor, and their responses to mechanical stimuli were quantified as the total number of paw withdrawals to an ascending series of calibrated von Frey filaments (.16, .4, 1.0, 2.0, and 4.0 g) applied to the plantar surface of the left hind paw. A positive response was withdrawal of the hind paw from the filament. Each von Frey filament was applied 5 consecutive times at approximately 3-second intervals, and the maximum possible withdrawal was 25 responses. Paw withdrawal latency (PWL) to radiant heat stimuli was measured using a plantar stimulator analgesia meter as the time it took until the animal removed its paw. Mice were placed in acrylic glass enclosures on a heated glass surface. Radiant heat was applied from below to the plantar surface of the left hind paw with a cutoff time of 20 seconds to prevent tissue damage. Baseline behavioral responses were measured prior to surgery and prior to infection with HSV-1 vectors. On the last day of testing (day 22), mice were deeply anesthetized and transcardially perfused for immunohistochemistry.

Immunohistochemistry

Immunohistochemical analysis was performed as previously reported.[36] Briefly, 2 weeks after virus infection, mice were deeply anesthetized with isoflurane and then transcardially perfused with .1 M phosphate-buffered saline followed by 4% paraformaldehyde in .1 M phosphate-buffered saline. Vertebral columns were removed and postfixed in 4% paraformaldehyde solution for 24 hours followed by isolation of lumbar

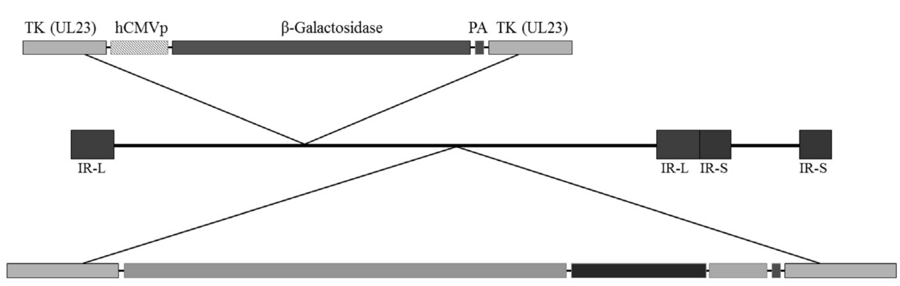

Figure 1. Schematic diagram of PZGalMORW (galMOR) recombinant herpes vector. Expression of β-galactosidase (*Escherichia coli* lacZ) is driven by the human CMV immediate-early enhancer promoter. Expression of the rat MOR is driven by the mouse 4.6-kb galanin promoter.[2] This transcription cassette contains a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) to enhance RNA stability. Abbreviations: PA, polyadenylation signal; IR, internal repeat; L, long; S, short.