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HSV-1-mediated NGF delivery delays nociceptive deficits in a genetic model of diabetic neuropathy

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Abstract

A previous phase III clinical trial failed to show significant therapeutic benefit of repeated subcutaneous nerve growth factor (NGF) administration in the treatment of diabetic neuropathy. Animal studies have since shown that site-specific viral-mediated expression of NGF in the lumbar dorsal root ganglia prevents peripheral nerve dysfunction associated with chemically induced neuropathy. Using a *Herpes simplex* virus expression vector, we have investigated the effect of localized NGF expression in a genetic mouse model of progressive diabetic neuropathy, the +/+ Leprdb mouse. We found that site-specific delivery of NGF initially delayed the appearance of hypoalgesia, assessed by the Hargreaves test, by 1 month and effectively attenuated this deficit for 2 months over the approximately 10 months normal life-span of these animals. Once the disease progressed into its more severe stages, NGF, although still capable of altering the electrophysiological profile of the sensory A- and C-fibers and influencing the expression of p75 and substance P in the dorsal root ganglia, could no longer maintain normal nociception. These data suggest that maximal therapeutic benefit in future NGF-based gene therapy trials will be gained from early applications of such viral-mediated neurotrophin delivery.

Keywords: Diabetic neuropathy; *Herpes Simplex* Virus-1; Nerve growth factor; Nociception; *Lepr db/db* mice

Introduction

Approximately half of the 5 million insulin-dependent and insulin-independent diabetic population in the USA suffer from peripheral neuropathy, the most common complication of diabetes (Dyck and Giannini, 1996; Dyck et al., 1993; Pirart et al., 1978). Diabetic neuropathy is a progressive disorder that initially affects the sensory neurons, causing a loss of nociception and proprioception progressing to affect the motor and autonomic neurons so leading to ataxia and autonomic failure (review Bird and Brown, 1996). When coupled with compromised vasculature, such polyneuropathies may lead to non-healing ulcers and ultimately lower limb amputation.

Nerve growth factor (NGF), one of a family of neurotrophins, supports the small diameter sensory neurons in the dorsal root ganglia (DRG) (Thoenen et al., 1987). This neurotrophic system is disturbed both in animal models of diabetic neuropathy and in diabetic patients (Anand, 1996; Pittenger and Vinik, 2003; Tomlinson et al., 1996b). Retrograde transport of NGF in the sciatic nerve is compromised and is accompanied by reduced NGF gene expression and protein levels in the skin and muscles (Fernyhough et al., 1994; Jakobsen et al., 1981; Tomlinson et al., 1997). The high and low affinity NGF receptors, trkA and p75, are also decreased in the DRG (Delcroix et al., 1997, 1998; Maeda et al., 1996). Administration of NGF reverses some of these changes (Delcroix et al., 1998), restores axonal conduction of some sensory neurons (Elias et al., 1998), peptide content in the DRG and spinal cord, and nociceptive behavior (Apfel et al., 1994; Schmidt et al., 1995), but does not affect cutaneous innervation (Christianson

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et al., 2003a). NGF is not alone in being disrupted: other neurotrophins such as neurotrophin-3 (NT-3) and glial-cell-line-derived neurotrophic factor (GDNF) are similarly affected (Akkina et al., 2001; Cai et al., 1999; Christianson et al., 2003b; Fernyhough et al., 1998; Tomlinson et al., 1996a).

Although these neurotrophic factors are clearly linked with peripheral diabetic neuropathy, it is unlikely that a dysfunction of the neurotrophins could alone account for the pathology of this disease. Nevertheless, the application of these neurotrophic factors as a rational approach to alter the degenerative pathology of specific primary afferent neurons in the treatment of diabetic neuropathy has been explored. Phase II clinical trials in which diabetic patients received repeated subcutaneous injections of NGF over 6 months showed a subjective improvement in symptoms (Apfel, 1999). However, the ensuing phase III clinical trial failed to confirm the earlier promise of NGF treatment in diabetic neuropathy, and the clinical development of NGF as a suitable treatment appears to be on hold (Apfel, 2002). The reasons for failure are likely multifactorial, but high on the list of possibilities is the dose and route of administration. Pre-clinical studies in chemically induced animal models of diabetes demonstrated beneficial effects of peripheral NGF administration in the 1–10 mg/kg range (Apfel et al., 1994; Diemel et al., 1994), whereas the phase III clinical trial employed a dose of 0.1 μg/kg. Furthermore, NGF was effective in animal models in preventing the progression rather than reversing the symptoms of neuropathy. The apparent lack of progression of symptoms in the placebo group over the 1-year time-span of the clinical trial may have precluded observation of any possible therapeutic benefit of NGF (Apfel, 2002).

Subsequent animal studies have shown encouraging benefits of site-specific expression of NGF or NT3, obtained by viral-mediated delivery to the lumbar primary afferent neurons, in two chemically induced models of neuropathy (Chattopadhyay et al., 2004; Goss et al., 2002). Such localized neurotrophic delivery enhanced nerve conduction velocity, increased neuropeptide content, and maintained proprioception. These encouraging data prompted us to further explore this approach using a mouse model of diabetes with a slower onset of peripheral neuropathy, as a closer model of the human condition. In this model, the +/+Leprdb mouse, expression of the long isoform of the leptin receptor is reduced so that leptin can no longer mediate satiety (Ghilardi et al., 1996). This leads to obesity, with diabetes arising as a secondary complication together with a concomitant slowly developing neuropathy (Robertson and Sima, 1980). We investigated how an NGF-expressing *Herpex simplex* virus-1 (HSV-1) vector, delivered to the lumbar DRG via sciatic nerve injection, affected thermal nociceptive behavior during the progression of the disease over the lifetime of these mice. In addition, the influence of this treatment on the electrophysiological properties of sensory neurons and on the expression of peptides within the DRG of +/+Leprdb and wild-type mice was also determined.

Materials and methods

Viruses

The cDNAs encoding the β-subunit of mouse NGF or β-Galactosidase (βGal) were placed behind two HSV-1 promoters in tandem orientation: the latency-associated transcript (LAT) and the long terminal repeat (LTR). This combination of promoters results in long-term expression of βGal in the DRG during the latent phase of the virus (Bloom et al., 1994; Lokensgard et al., 1997). These expression constructs were recombined into the HSV-1 genome, replacing the ICP4 locus and generating a virus that can no longer replicate. The resultant recombinant viruses (Fig. 1) were plaque-purified and amplified using the E5 helper cell line. Stocks were spun through a Nycodenz gradient (Greiner Bio-One, Longwood, FL) to generate pure recombinant viral stocks of 3×10^8 (HSV-NGF) and 4×10^8 (HSV-βGal) plaque-forming units (pfu)/ml.

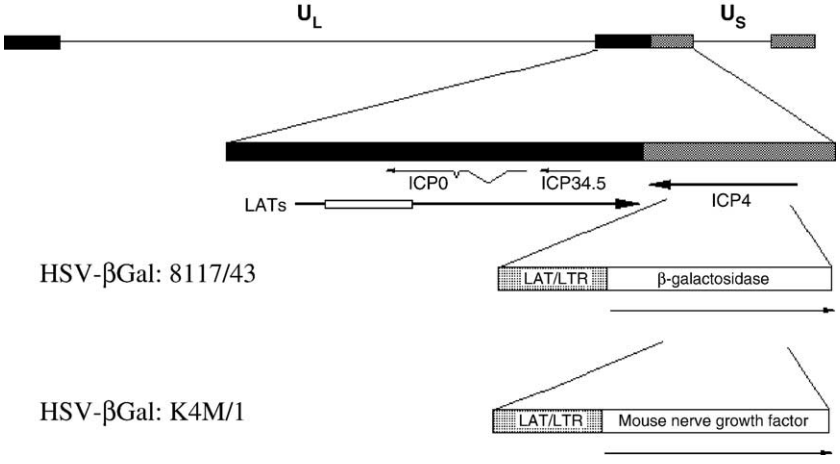

Fig. 1. Viral characterization. A schematic diagram of HSV-NGF and HSV-βGal showing the insertion of the LAT-LTR-NGF/-βGal expression cassette within the ICP4 locus of the HSV-1 genome. Shown at the top is the HSV-1 genome and below an expanded view of the Long and Short internal repeat regions (shown as black and gray boxes, respectively). The LAT/LTR βGal or NGF cassettes were inserted in place of the ICP4 gene as previously described (Dobson et al., 1990; Bloom, 1996) such that both copies of the ICP4 gene (both in the internal and terminal Short repeats) were replaced.