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Decrease in neuroimmune activation by HSV-mediated gene transfer of TNFα soluble receptor alleviates pain in rats with diabetic neuropathy

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ABSTRACT

The mechanisms of diabetic painful neuropathy are complicated and comprise of peripheral and central pathophysiological phenomena. A number of proinflammatory cytokines are involved in this process. Tumor necrosis factor α (TNF-α) is considered to be one of the major contributors of neuropathic pain. In order to explore the potential role of inflammation in the peripheral nervous system of Type 1 diabetic animals with painful neuropathy, we investigated whether TNF-α is a key inflammatory mediator to the diabetic neuropathic pain and whether continuous delivery of TNFα soluble receptor from damaged axons achieved by HSV vector mediated transduction of DRG would block or alter the pain perception in animals with diabetic neuropathy. Diabetic animals exhibited changes in threshold of mechanical and thermal pain perception compared to control rats and also demonstrated increases in TNFα in the DRG, spinal cord dorsal horn, sciatic nerve and in the foot skin, 6 weeks after the onset of diabetes. Therapeutic approaches by HSV mediated expression of p55 TNF soluble receptor significantly attenuated the diabetes-induced hyperalgesia and decreased the expression of TNFα with reduction in the phosphorylation of p38MAPK in the spinal cord dorsal horn and DRG. The overall outcome of this study suggests that neuroinflammatory activation in the peripheral nervous system may be involved in the pathogenesis of painful neuropathy in Type 1 diabetes which can be alleviated by local expression of HSV vector expressing p55 TNF soluble receptor.

1. Introduction

Painful neuropathy is a common, difficult to treat complication of diabetes. The pathogenesis of diabetic neuropathy is complex and involves multiple pathways. Lack of success in preventing neuropathy even with successful treatment of hyperglycemia suggests the presence of early mediators between hyperglycemia-induced metabolic and enzymatic changes in functional and structural properties of the nervous system. Metabolic changes induced by hyperglycemia lead to dysregulation of cytokines which can cause pain-related symptoms (Skundric and Lisak, 2003). Approximately 20–24% of diabetes patients experience neuropathic pain (Schmader, 2002). Increasing evidence shows that inflammatory cytokines may participate in the pathogenesis of insulin resistance and its complications (Fernandez-Real and Ricart, 2003). Subjects with Type 1 diabetic (T1D) neuropathy have shown an increase in plasma concentrations of inflammatory markers that are involved in insulin resistance (Goldberg, 2009; Gonzalez-Clemente et al., 2007). Increased level of tumor necrosis factor alpha (TNFα) is often demonstrated in patients with diabetic neuropathy, regardless of their glycemic control and cardiovascular risk factors that are associated with insulin resistance (Averill and Bornfeldt, 2009; King, 2008). Patients with painful neuropathy have shown increased IL-2 and TNFα mRNA and protein levels in blood (King, 2008). Studies have shown that patients with painful diabetic neuropathy (PDN) exhibit a different serum immune profile compared to patients with painless diabetic neuropathy which suggests that immune markers in blood are associated with diabetic neuropathic pain (Doupis et al., 2009; Uceyler et al., 2007). Results from these studies suggest that TNFα may play a pathogenic role in the development of diabetic neuropathy (Gonzalez-Clemente et al., 2005). Majority of these studies have been done

Abbreviations: PDN, painful diabetic neuropathy; T1D, Type 1 diabetes; DRG, dorsal root ganglia; IL-2, interleukin-2; TNF, tumor necrosis factor; TNFsR, soluble TNF receptors.

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in Type 2 diabetic subjects using serum immune profiles. It is unclear whether similar mechanisms exist in Type 1 diabetes. The relationship of these serum inflammatory markers to nociceptive pathways in the nervous system in T1D animals has not been explored. We undertook these studies to understand whether TNFα, a major contributor in other forms of neuropathic pain, plays a role in this process and that whether increases in TNFα in the peripheral nervous system of T1D animals are responsible for painful neuropathy. TNFα is a pleiotropic cytokine which mediates the inflammatory response and activation of the immune system (Marchand et al., 2009). The effects of TNFα are mediated by 2 specific cell surface receptors, 55-kD TNF receptor I and 75-kD TNF receptor II. The extracellular domains of these receptors are released by proteolytic cleavage to form soluble TNF (sTNF) receptor I and II (Horiuchi et al., 2010). Soluble TNF receptors act as TNF antagonists and inhibit TNFα-mediated proinflammatory effects.

A broad range of preclinical animal studies have shown that systemic delivery of neurotransmitters or anti-inflammatory agents could effectively prevent painful neuropathy. Unfortunately, administration of these agents in patients has resulted in a number of systemic side effects. Currently available treatments to effectively treat or prevent the progression of painful neuropathy are very limited. Earlier studies have shown that pretreatment with intra-peritoneal administration of etanercept, a commercially available soluble human 75-kD TNF receptor, reduces mechanical allodynia in rats with the spinal nerve ligation or spinal cord hemi section model of peripheral neuropathic pain (Marchand et al., 2009; Schafers et al., 2003). In diabetic mice, both systemic or intrathecal administration of etanercept produced only a dose dependent reversal of tactile allodynia but not thermal hyperalgesia (Dogrul et al., 2011). This suggests that a better therapeutic approach is needed. To avoid systemic side effects, local expression of anti-inflammatory agent may overcome this consequence. Our study demonstrates that subcutaneous injection of replication defective HSV based vectors in the footpad to deliver and express the transgenes in primary sensory neurons of the DRG may prevent the progression of this condition. Previous studies have shown that footpad inoculation of HSV vector expressing p55TNF soluble receptor one week before nerve injury reduced mechanical allodynia and thermal hyperalgesia, and also resulted in a reduction of TNFα and concomitant reductions in interleukin (IL)-1β and phosphorylated p38 MAP kinase as well (Hao et al., 2007). Another spinal cord injury model of peripheral neuropathic pain involving footpad inoculation of HSV vector expressing p55TNF soluble receptor one week after injury showed alleviation of mechanical allodynia (Peng et al., 2006). In this study we investigated that continuous delivery of TNFα soluble receptor from damaged axons achieved by HSV vector mediated transduction of DRG in animals with Type 1 diabetes, blocks the nociceptive and stress responses in the DRG and spinal dorsal horn neurons by reducing the TNFα expression and also decreasing the downstream signaling molecule p38MAPK activation with improved pain perception. Additionally we found that the increases in TNFα in sciatic nerve and in the foot skin are also reduced in animals treated with the therapeutic vector in conjunction with the reduction of pain. Taken together these studies suggest that neuroinflammatory activation in the peripheral nervous system may be involved in the pathogenesis of painful neuropathy in Type 1 diabetes.

2. Materials and methods

2.1. Animal studies: induction of diabetes and vector inoculation

Experiments were performed on male Sprague Dawley rats weighing 220–250 gms (Charles River, Portage, MI, USA) in compliance with approved institutional animal care and use protocols (IACUC, VAAAHS). Animals were rendered diabetic by IP injection of streptozotocin (STZ; 50 mg/kg). Three days after STZ injections, blood glucose levels were measured using OneTouch Ultra glucose meter (LifeScan, Inc; USA). Rats with greater than 300 mg/dl blood glucose level were included in the study (Table 1). Two weeks after the onset of diabetes, the T1D animals were inoculated with either HSV vector expressing p55 TNFα soluble receptor (vTNFsR) or control vector expressing *lac*Z (vZ) subcutaneously into the plantar surface of both hind paw (30 μl of 1×10^9 pfu/ml). Animals were grouped into four categories: control, diabetic alone, diabetic inoculated with vTNFsR (dia + vTNFsR) and diabetic inoculated with vZ (dia + vZ). All behavioral analyses were carried out by an observer blinded to the treatment group 4 weeks after vector inoculation (at 6 weeks of diabetes). A group of diabetic animals were evaluated at 2 weeks after hyperglycemia to assess the pain related behaviors at the time of vector treatment. A separate group of rats were injected in a similar fashion with vTNFsR and euthanized 7 days later for the studies of transgene expression *in vivo*.

2.2. Behavioral studies

2.2.1. Thermal hyperalgesia

The latency to hind paw withdrawal from a thermal stimulus was determined by exposing the plantar surface of the hind paw to radiant heat using a modified Hargreaves (Hargreaves et al., 1988) thermal testing device. Rats were placed in individual enclosures on a glass plate maintained at 30 °C, and after a 30 min habituation period the plantar surface of the paw was exposed to a beam of radiant heat applied through the glass floor. Activation of the bulb simultaneously activated a timer, and both were immediately turned off by paw withdrawal or at the 20 s cut-off time. Testing was performed by a blinded observer in triplicate at 5 min intervals.

2.2.2. Mechanical hyperalgesia

Mechanical nociceptive threshold was assessed using an analgesimeter (Ugo Basile, Comerio, VA, Italy) as described by Randall and Selitto (1957). A linearly increasing pressure was applied through a cone-shaped plastic tip with a diameter of 1 mm onto the dorsal surface of the hindpaw. The tip was positioned between the third and fourth metatarsus, and force applied until the rat attempted to withdraw its paw (paw withdrawal threshold to pressure). The pain threshold determined as the mean of three consecutive stable values expressed in grams was determined by a blinded observer.

2.3. Cell culture experiment

DRG from adult rats were dissociated with 0.25% trypsin, 1 mM EDTA for 30 min at 37 °C with constant shaking and then plated on poly-D-lysine-coated coverslips (10^5 cells per well in a 24-well

Table 1
Changes in metabolic parameters before and after the onset of diabetes. Control and diabetic animals gained weight significantly ($^{***}P < 0.001$) 6 weeks after the onset of diabetes compared to their pre-diabetic states. Animals treated with STZ have increased blood glucose level compared to the control animals ($^{\#}P < 0.005$) as measured 6 weeks post-diabetes.

	Pre-diabetic		6 weeks post-diabetic	
	Weight (gm)	Glucose (mg/dL)	Weight (gm)	Glucose (mg/dL)
Control	247.5 ± 9.1	114.3 ± 6.3	304 ± 10.2***	121.5 ± 11.4
STZ-diabetic	251.1 ± 6.5	118 ± 5.4	299.3 ± 9.2***	401.8 ± 50.8$^{\#}$

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