Molecular and Cellular Neuroscience 72 (2016) 46–53

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

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

Viral vector mediated continuous expression of interleukin-10 in DRG alleviates pain in type 1 diabetic animals

Vikram Thakur, Mayra Gonzalez, Kristen Pennington, Munmun Chattopadhyay *

Center of Excellence in Diabetes and Obesity, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, TX, United States

ARTICLE INFO

Article history:
Received 3 August 2015
Revised 3 December 2015
Accepted 15 January 2016
Available online 21 January 2016

Keywords:
Diabetic neuropathy
Pain
Interleukin-10
DRG
Gene therapy
Inflammation, TLR4

ABSTRACT

Painful diabetic neuropathy is a common and difficult to treat complication of diabetes. A growing body of evidence implicates the role of inflammatory mediators in the damage to the peripheral axons and in the pathogenesis of neuropathic pain. Increased expression of pro-inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α in the peripheral nervous system suggests the possibility of change in pain perception in diabetes. In this study we investigated that continuous delivery of IL10 in the nerve fibers achieved by HSV vector mediated transduction of dorsal root ganglion (DRG) in animals with Type 1 diabetes, blocks the nociceptive and stress responses in the DRG neurons by reducing IL1β expression along with inhibition of phosphorylation of p38 MAPK (mitogen-activated protein kinase) and protein kinase C (PKC). The continuous expression of IL10 also alters Toll like receptor (TLR)-4 expression in the DRG with increased expression of heat shock protein (HSP)-70 in conjunction with the reduction of pain. Taken together, this study suggests that macrophage activation in the peripheral nervous system may be involved in the pathogenesis of pain in Type 1 diabetes and therapeutic benefits of HSV mediated local expression of IL10 in the DRG with the reduction of a number of proinflammatory cytokines, subsequently inhibits the development of painful neuropathy along with a decrease in stress associated markers in the DRG. This basic and preclinical study provides an important evidence for a novel treatment strategy that could lead to a clinical trial for what is currently a treatment resistant complication of diabetes.

1. Introduction

Painful neuropathy is a common complication of diabetes. The pathogenesis of pain in diabetic neuropathy is not clear (Wunderlich et al., 1998; Calcutt, 2002, Schmader, 2002, Simmons and Feldman, 2002). Inflammatory or nociceptive pain results from tissue damage to either somatic or visceral structures resulting in activation of peripheral nociceptors, whereas neuropathic pain is caused by peripheral nerve injury or dysfunction in the absence of tissue injury. In both nociceptive and neuropathic pain conditions, a converging body of evidence suggests that the activation of supporting cells (microglial, satellite and Schwann cells) (Raghavendra et al., 2003; Zhuang et al., 2005) and expression of bioactive substances may play a role in immune response (Cui et al., 2000; Lindenlaub and Sommer, 2003). We and others have previously shown that inflammation in peripheral nervous system of Type 2 diabetic animals leads to increased pain compared with those from age-matched controls (Cai, 2013, Galloway and Chattopadhyay, 2013). However, there is not enough data on macrophage activation and inflammation in the peripheral nervous system leading to pain in type 1 diabetes. The main objective of this study was to assess the role of Herpes Simplex viral (HSV) vector mediated continuous expression of anti-inflammatory cytokine interleukin-10 (IL10) in relieving pain in diabetic neuropathy and also to explore the activation of inflammatory cascade in the peripheral nervous system. The neuronal dysfunction in diabetes has been closely associated with the activation of nuclear factor-kappa B (NF-κB) (Bierhaus et al., 2001, 2004). Therefore, increased expression of pro-inflammatory cytokines, such as, interleukin (IL)-1β and tumor necrosis factor (TNF)-α suggests the possibility of change in pain perception in diabetes. To control the pain due to alteration in inflammatory cascade, this study investigates the role of anti-inflammatory cytokine IL10 in alleviating this 'neuroimmune' response. Interleukin-10 (IL-10) has been shown to have an anti-nociceptive effect in central and peripheral nervous systems (Wagner et al., 1998; Froen et al., 2002). It prevents the activation of microglia and suppresses the production of pro-inflammatory cytokines in other injury models of neuropathic pain (Milligan et al., 2005a, 2006). In the current study, we explicitly tested the role of neuroimmune activation in the pathogenesis of pain in rats with diabetes and examined the application of gene transfer of IL-10 with a non-replicating herpes simplex virus (HSV)

Abbreviations: T1D, type 1 Diabetes; DRG, dorsal root ganglia; IL-10, interleukin-10; TNF, tumor necrosis factor; TLR4, toll like receptor 4; HSP70, heat shock protein 70.

* Corresponding author at: Department of Biomedical Sciences, Center of Excellence in Diabetes and Obesity, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, TX, United States.
E-mail address: munmun.chattopadhyay@ttuhsc.edu (M. Chattopadhyay).

http://dx.doi.org/10.1016/j.mcn.2016.01.006
1044-7431/

based vector to alleviate the process. We investigated that continuous delivery of IL10 in the nerve fibers achieved by HSV vector mediated transduction of dorsal root ganglion (DRG) in animals with Type 1 diabetes, blocks the nociceptive and stress responses in the DRG neurons by reducing the IL1β expression with inhibition of phosphorylation of p38 MAPK (mitogen-activated protein kinase) and protein kinase C (PKC). We also found that the increases in Toll like receptor (TLR)-4 in the DRG are reduced in therapeutic vector treated animals with increased expression of heat shock protein (HSP)-70 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 and demonstrate the therapeutic benefits of HSV mediated local expression of IL10 in the DRG with the reduction of a number of proinflammatory cytokines, subsequently impeding the development of neuropathy along with a decrease in stress associated markers in the DRG.

2. Materials and methods

2.1. Animal studies: diabetes development and vector delivery

Male Sprague Dawley rats (SD; Charles River, USA) were used to conduct these experiments, in compliance with approved institutional animal care and use protocols. Animals were made 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 blood glucose level higher than 300 mg/dl were included in the study. Two weeks after the blood glucose measurement, rats were inoculated subcutaneously into the plantar surface of both hind paw (30 μl of 1×10^9 pfu/ml) with either HSV vector expressing Interleukin-10 (vIL-10; a gift from Dr. David Fink, University of Michigan) or a control vector expressing *lacZ* (vZ). All behavioral studies were carried out 4 weeks after vector inoculation (6 weeks of diabetes) by an observer blinded to the treatment and control groups. Previously we have demonstrated that the behavioral and other signaling data were not different in diabetic versus control *lacZ* vector treated diabetic animals (Ortmann and Chattopadhyay, 2014). We have also reported earlier that 40–50% of DRG neurons are transfected after footpad inoculation (Chattopadhyay et al., 2012). In this study we compared the groups between diabetic-therapeutic and diabetic-control vector treated groups.

2.2. Behavioral studies

2.2.1. Thermal hyperalgesia

Using a modified Hargreaves (Hargreaves et al., 1988) thermal analysis device, hind paw withdrawal latency (measured in seconds), was determined by exposing the plantar surface of the hind paw to a thermal stimulus of beaming heat from a bulb. Rats were placed in individual enclosures for 30 min, as a habituation period, on the glass surface of the device which was maintained at 30 °C. The plantar surface of the paw was exposed to a beam of radiant heat applied through the glass floor. Paw withdrawal threshold by the animal due to heat, or the 20 s cut-off time, turned off the radiant bulb and timer simultaneously. Testing was performed by a blinded observer in triplicate with a 5 min interval.

2.2.2. Mechanical hyperalgesia

Mechanical nociceptive threshold was measured using an analgesimeter (Ugo Basile, Comerio, VA, Italy). A 1 mm diameter cone shaped plastic tip was used to apply a linearly increasing pressure onto the dorsal surface of the hind paw. 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). Three consecutive stable values expressed in grams were determined by a blinded observer, and then the pain threshold was determined by the mean value of these three numbers.

2.3. Western blot

For each control and IL-10 vector treated rat, samples of DRG (L4, L5, and L6) were collected. These tissues were homogenized with lysis buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10% glycerol and 1:100 dilution of Protease Inhibitor Cocktail, and Phosphatase Inhibitor Cocktail (Sigma, St. Louis, MO). The homogenized tissues were centrifuged for 10 min at 10,000 g at 4 °C and the supernatant was stored at −80 °C. Protein estimation of the supernatant was done using a protein assay kit (Bio-Rad Laboratories, Hercules, CA) and aliquots of 20 μg of protein per lane were made. These proteins were separated by PAGE, transferred to a PDVF membrane (0.45 μm, Thermo Scientific, Waltham, MA), blocked with 5% nonfat milk, then incubated with the primary antibody (P-P38 1:300, IL1β 1:300 [Santa Cruz Biotechnology, Santa Cruz, CA]; PPKC-α/β 1:500 and TLR4 1:300 [Cell Signaling, Danvers, MA]; ED1 1:400 and HSP70 1:500, [Millipore, Bedford, MA]. Then membranes were incubated in horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG (1:2000 Amersham, Piscataway, NJ) and visualized with Enhanced Chemiluminescence (ECL, Pierce, Rockford, IL). And as a loading control, the membranes were reprobed with β-actin (1:2000; Sigma). The intensity of each band was determined by quantitative chemiluminescence using the ChemiDoc XRS System, a PC-based image analysis system (Bio-Rad Lab., USA) and normalized to the respective level of β-actin.

2.3.1. 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 plate) in 500 μl of defined Neurobasal media containing B27, GlutaMAX I, Albumax II, and penicillin/streptomycin (Gibco-BRL, Carlsbad, CA), supplemented with 100 ng/ml of 7.0S NGF per ml (Sigma, St Louis, MO). 5-Fluoro-2′-deoxyuridine (5 μM; Sigma) and uridine (5 μM; Sigma) were added on days 1 and 3 to inhibit the growth of dividing cells. After 3 days in vitro, cells were exposed to hyperglycemic condition overnight; the medium was changed to 25 mM glucose (in addition to the basal glucose) with GlutaMAX I, Albumax II, penicillin/streptomycin, but without B27 and NGF (cells are viable without NGF at this point). Control cells were exposed to an identical medium without B27 and NGF but with basal glucose. For the TLR4 antagonist experiment, cells were exposed to 5 μM of TAK242 (EMD Millipore, USA) overnight. After 16 h of exposure, cells were collected in cell lysis buffer and Western blot was performed.

2.3.2. Immunohistochemistry

Half of the rats from the study were transcardially perfused with 0.9% NaCl followed by 500 mL of Zamboni's fixative (Verdu et al., 1999). The DRG were removed, post-fixed with Zamboni's fixative for 2 h, and then cryo-protected with 30% sucrose in phosphate buffered saline (PBS) overnight, cryostat sectioned at 10 μm and collected on microscope slides. Tissue sections were fixed with 4% PFA (Paraformaldehyde) for 45 min and washed with PBS three times. Then incubated with 1% Sodium Borohydrate ($NaBH_4$) for aldehyde blocking and washed three times with PBS. After this the tissue sections were incubated with blocking solution (PBS with 1% normal goat serum, 0.3% Triton X-100 and 1000X NaAZide) for 1 h, then washed once before being incubated with the primary antibody IL1β 1:400, ED1 1:500, S-100β 1:500 (Santa Cruz Biotechnology, Santa Cruz, CA), overnight at 4 °C and washed three times on the next day. After incubation in the secondary fluorescent antibody, Alexa Fluor 594 goat anti-rabbit IgG 1:500 or Alexa Flour 488 goat anti-mouse IgG 1:500, respectively (Life Technologies, Carlsbad, CA) for 2 h in the dark at room temperature. Lastly the specimens were washed 3 times and incubated in DAPI