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MURINE MODEL AND MECHANISMS OF TREATMENT-INDUCED PAINFUL DIABETIC NEUROPATHY

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Abstract—Diabetes mellitus represents a group of metabolic diseases that are characterized by hyperglycemia caused by either lack of insulin production or a reduced ability to respond to insulin. It is estimated that there were 347 million people worldwide who suffered from diabetes in 2008 and incidence is predicted to double by 2050. Neuropathy is the most common complication of long-term diabetes and approximately 30% of these subjects develop chronic neuropathic pain. A distinct acute, severe form of neuropathic pain, called insulin neuritis or treatment-induced painful neuropathy of diabetes (TIND), may also occur shortly after initiation of intensive glycemic control, with an incidence rate of up to 10.9%. The pathological mechanisms leading to TIND, which is mostly unresponsive to analgesics, are not yet understood, impeding the development of therapies. Studies to date have been clinical and with limited cohorts of patients. In the current study, we developed chronic and acute insulin-induced neuropathic pain in mice with type 2 insulin-resistant diabetes. Furthermore, we determined that insulin-induced acute allodynia is independent of glycemia levels, can also be induced with Insulin-like Growth Factor 1 (IGF1) and be prevented by inhibition of AKT, providing evidence of an insulin/IGF1 signaling pathway-based mechanism for TIND. This mouse model is useful for the elucidation of mechanisms contributing to TIND and for the testing of new therapeutic approaches to treat TIND.

Key words: diabetes, neuropathy, insulin neuritis, insulin, TIND.

INTRODUCTION

Diabetes mellitus comprises a group of metabolic diseases that are characterized by hyperglycemia caused by either lack of insulin production or a reduced ability to respond to insulin. About 347 million people worldwide were estimated to suffer from diabetes in 2008 and incidence is predicted to double by 2050 (Danaei et al., 2011). The majority of cases represent type 2 diabetes, whereas 5–10% of diabetic patients suffer from type 1 diabetes. In both forms, neuropathy is the most common complication of long-term diabetes and approximately 30% of such subjects develop chronic neuropathic pain (Abbott et al., 2011).

A distinct, acute, severe form of neuropathic pain may also occur shortly after initiation of intensive glycemic control. First described by Caravati in 1933, the condition was considered a rare iatrogenic neuropathy (Caravati, 1933) and its mechanisms are still unknown. This neuropathy is sometimes referred to as insulin neuritis or acute painful diabetic neuropathy of rapid glycemic control, but has most recently been classified as treatment-induced painful neuropathy of diabetes (TIND) (Tesfaye et al., 1996; Knopp et al., 2013; Gibbons and Freeman, 2015). Both type 1 and type 2 diabetic patients experience TIND, which develops following onset of treatment with insulin (Gibbons and Freeman, 2015). While TIND occurred following treatment with oral hypoglycemic agents, it is reported with less frequency (Leow and Wyckoff, 2005; Knopp et al., 2013). Previous studies originally reported incidences to be around 1%, however, a more recent, extensive clinical study has found that up to 10.9% of study patients met the criteria for TIND (Gibbons and Freeman, 2015). The number of patients suffering from TIND is prone to increase with the predicted doubling of the diabetic population (Danaei et al., 2011; Gibbons and Freeman, 2015) and the recommendation of rigorous glycemic control implemented following the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) studies (DCCT research group, 1988, Martin et al., 2006, Albers et al., 2010).

TIND is characterized by allodynia and/or burning painful paresthesias and may occur distally in a length-dependent fashion or be more generalized and involved proximal sites including the trunk (Archer et al., 1983; Tesfaye et al., 1996; Dabby et al., 2009; Gibbons and Freeman, 2010). Patients start to experience excruciating neuropathic pain within 2–4 weeks of treatment initiation (Dabby et al., 2009). This pain is frequently described

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Abbreviations: HFD, high-fat diet; IGF1, Insulin-like Growth Factor 1; IR-KD, inducible insulin receptor knockdown; LFD, low-fat diet; MNCV, motor nerve conduction velocity; PWT, 50% paw withdrawal threshold; TIND, treatment-induced painful neuropathy of diabetes.

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as severe and, in one study, is rated 10 on a scale of 1–10 by all subjects (Gibbons and Freeman, 2010), despite being treated with multiple medications. The pain phenotype can vary and it is particularly notable that allodynia and burning pain are experienced after insulin therapy, while allodynia is not experienced after instituting glycemic control using oral hypoglycemic agents (Dabby et al., 2009). TIND is refractory to currently available therapeutic interventions (Dabby et al., 2009; Gibbons and Freeman, 2010), including all of the first line treatments for "classic" chronic painful diabetic neuropathy such as tricyclic antidepressants, anticonvulsants or serotonin reuptake inhibitors. In some case reports, pain persisted, and even increased, despite use of analgesics and sedatives, with cessation of insulin treatment being the only approach to alleviating pain – but leading to severe hyperglycemia (Caravati, 1933). While the disease is resistant to treatment with multiple analgesics (Knopp et al., 2013), the condition subsides within weeks to months for most patients, but can last years (Caravati, 1933; Gibbons and Freeman, 2010).

The pathological mechanisms leading to TIND are not understood, which impedes the development of targeted therapies. The currently proposed theories are endoneurial ischemia, hypoglycemic microvascular damage and regenerating nerve firing (Llewelyn et al., 1986; Tesfaye et al., 1996; Gibbons and Freeman, 2010). It has been suggested that institution of good glucose control, or perhaps insulin per se, may promote axonal regeneration that causes pain sensations. Prominent regenerative activity in the absence of active degeneration was observed in patients suffering from TIND and pain may possibly result from ectopic generation of impulses in the regenerating axon sprouts (Archer et al., 1983; Llewelyn et al., 1986). More recently, peripheral nerve degeneration described as diffuse damage to unmyelinated and lightly myelinated nerve fibers and a decrease in intra-epidermal nerve fibers that was temporally related to the rapid improvement in glucose control were observed in patients suffering from TIND (Gibbons and Freeman, 2010). TIND could also result from changes in nerve vasculature. Proliferating epineurial blood vessels, similar to those found in retinopathy, have been described in nerve of patients suffering from TIND. Some patients showed severe abnormalities, such as arterial attenuation and arterial-venous shunting that could lead to endoneurial ischemia (Tesfaye et al., 1996). Similarly, insulin was shown to reduce nerve nutritive blood flow and increase arterio-venous shunt flow when administered by intravenous infusion at doses from 0.04U/kg up to 32U/kg (Kihara et al., 1994). However, normal vasa nervosum was observed in other patients suffering from TIND (Archer et al., 1983; Vital et al., 1997).

Studies to date are clinical with limited cohorts of patients, in part because of the lack of awareness about the disorder. Establishing an animal model becomes necessary to study the mechanism of TIND and potential therapeutics. In this study, we developed a protocol for acute and chronic insulin-induced neuropathic pain in mice with type 2 insulin-resistant diabetes. Furthermore, we determined that insulin-induced acute allodynia is independent of glycemia levels, can also be induced through Insulin-like Growth Factor 1 (IGF-1) and can be alleviated by an AKT inhibitor, providing evidence of an insulin/IGF1 signaling pathway-based mechanism for TIND.

EXPERIMENTAL PROCEDURES

Animals and diet

Adult female C57Bl6 mice were purchased from Harlan Industries/Envigo (Placentia, CA, USA). Inducible insulin receptor knockdown (IR-KD) mice were purchased from Taconic (USA) and male db/db (5 months old, BKS.Cg-*Dock7*^{m}+/+ *Lepr*^{db}/J) mice were purchased from Jackson Laboratory (USA). A total of 74 animals were housed 3–4 per cage with free access to food and water and maintained in a vivarium approved by the American Association for the Accreditation of Laboratory Animal Care. All animal studies were carried out according to protocols approved by the Institutional Animal Care and Use Committee of the University of California San Diego. Six to 8 mice were used per group. C57Bl6 mice were fed a low-fat diet (LFD, 10% kcal from fat, Research Diet Inc., New Brunswick, NJ, USA) or a high-fat diet (HFD, 60% kcal from fat, Research Diet Inc., New Brunswick, NJ, USA) up to 33 weeks. IR-KD mice were fed a regular diet but were maintained on doxycycline water to induce a reduction of insulin receptor expression (Seibler et al., 2007). db/db mice were fed a regular diet.

Chronic insulin-induced painful neuropathy. After 14 weeks of HFD regimen, once insulin resistance was confirmed by glucose tolerance test, a group of HFD mice was treated with 1U insulin (Humulin R) subcutaneous injections five times a week between 9 and 11 am. HFD regimen and insulin injection continued for 8 weeks. Tactile allodynia and responses to thermal stimuli were measured 5-6 h and 6-7 h, respectively, after insulin injection (unless otherwise specified) once a week.

Acute insulin-induced painful neuropathy

After 15 weeks of HFD regimen, once insulin resistance was confirmed by glucose tolerance test, other groups of HFD mice received a single dose of saline, insulin (0.5 or 2.0U) or IGF1 (2 mg/kg, Prospec Inc., NJ, USA) sc injection and tactile allodynia was assessed every 30 min for 210 min.

Glucose tolerance test

To assess insulin resistance, glucose was administered at 1.5 g/kg ip after on overnight fast. Glucose levels were measured using a strip-operated reflectance meter in a blood sample obtained by tail prick every 30 min for 2 h.

Tactile allodynia. Impaired sensation to touch is one of the most common pain reported by patients suffering from