Available online at www.sciencedirect.com
ScienceDirect
Experimental Neurology 207 (2007) 23–29
Experimental Neurology
www.elsevier.com/locate/yexnr

The neurocytokine, interleukin-6, corrects nerve dysfunction in experimental diabetes

Norman E. Cameron *, Mary A. Cotter

School of Medical Sciences, College of Life Sciences and Medicine, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK

Received 13 March 2007; revised 11 May 2007; accepted 11 May 2007
Available online 18 May 2007

Abstract

Interleukin-6 (IL-6) is a member of the neuropoietic cytokine family and has a multifunctional biological role in regulating the immune response, acute phase reactions, and hematopoiesis. IL-6 is also important in neural development and has neurotrophic actions. The aim was to ascertain whether IL-6 treatment could rectify some of the adverse early changes in neurovascular function in streptozotocin-induced diabetic rats. After 4 weeks of untreated diabetes, rats were treated with IL-6 (1–10 μg/kg thrice weekly) for 4 weeks. Diabetes caused 22% and 22.5% reductions in sciatic nerve motor and saphenous nerve sensory conduction velocity, respectively, which were dose dependently corrected by treatment. Diabetic rats also showed thermal hyperalgesia and tactile allodynia, which were completely corrected by IL-6; however, IL-6 was ineffective against mechanical hyperalgesia. Sciatic nerve endoneurial perfusion was 42.2% reduced by diabetes and blood flow was returned to the nondiabetic range by 10 μg/kg IL-6 treatment. The ED_{50} values for these actions ranged from 1.2 μg/kg for sensory conduction velocity to 3.2 μg/kg for sciatic nerve perfusion. Thus, IL-6 treatment improved several measures of nerve dysfunction in experimental diabetes, and these effects correlated with a recovery of nerve blood flow. The magnitude of these beneficial effects and the potential joint neurotrophic and vascular action suggests that IL-6 could be a candidate for further evaluation in clinical trials of diabetic neuropathy.

Keywords: Diabetes; Neuropathy; Interleukin-6; Nerve conduction; Blood flow; Pain; Allodynia

Introduction

Diabetes mellitus is a major cause of peripheral neuropathy; the etiology is associated with changes in multiple metabolic and vascular mechanisms (Cameron et al., 2001a). All nerve fiber types are affected. Circulating levels of the cytokine, interleukin-6 (IL-6), are elevated in insulin-resistant states such as type 2 diabetes and, along with raised C-reactive protein and tumor necrosis factor (TNF)-α levels, this has been considered a surrogate marker of low-grade chronic inflammation that may play a pathogenetic role (Sjoholm and Nystrom, 2006). However, the interrelations between cytokines are complex, and while a good case may be made for involvement of TNF-α in type 2 diabetes, the elevated IL-6 levels may be correlative rather than causative (Carey and Febbraio, 2004). Indeed, IL-6 antagonizes TNF-α production; and IL-6 levels are increased by exercise which improves insulin sensitivity (Pedersen et al., 2004). Furthermore, IL-6 knockout mice develop insulin resistance (Wallenius et al., 2002). Thus, IL-6 may have metabolic benefits, at least in type 2 diabetes, and in the context of neuropathy there are potentially valuable neurotrophic properties.

IL-6 is a multifunctional member of the neuropoietic cytokine family, which includes ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and cardiotrophin-1 (Gadient and Otten, 1997; Taga and Kishimoto, 1997; Skundric and Lisak, 2003). IL-6 supports neuronal survival, reducing neurotoxicity in vitro (Hama et al., 1989; Yamada and Hatanaka, 1994; Akaneya et al., 1995), and has in vivo neuroprotective actions against insults such as axotomy, ischemia–reperfusion, and viral-induced demyelination (Rodriguez et al., 1994; Ikeda et al., 1996; Matsuda et al., 1996). In IL-6-deficient knockout mice, there is delayed axonal regeneration

* Corresponding author. Fax: +44 1224 555719.
E-mail address: n.e.cameron@abdn.ac.uk (N.E. Cameron).

0014-4886/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2007.05.009

and impaired sensory function; on the other hand, transgenic mice overexpressing IL-6 show improved nerve regenerative capacity (Hirota et al., 1996; Zhong et al., 1999). A recent study demonstrated that IL-6 treatment partially prevents the development of delayed motor distal latency and thermal hypoalgesia in a diabetic rat model (Andriambeloson et al., 2006). In addition to neuronal actions, IL-6 may also modulate vascular function (Vila and Salaices, 2005), which could be potentially important for some abnormalities of nerve function in diabetes, such as reduced conduction velocity. Circulating IL-6 levels tend to increase in streptozotocin-induced diabetic in rats (Dixon et al., 2007; Ugochukwu and Figgers, 2007), but this alone does not prevent neurovascular deficits from developing. Streptozotocin induction models type 1 diabetes, with a relative insulin deficiency which is metabolically less complex than changes contributing to the insulin resistance seen in type 2 models. Thus streptozotocin–diabetes provides a relatively simple model in which to investigate the trophic and related actions of IL-6 in experimental neuropathy. The aim was to examine the effects of pharmacological doses of IL-6 treatment in dose-response studies on nerve conduction velocity (NCV), sensory function, and nerve perfusion in streptozotocin-diabetic rats.

Methods

Animals and experimental design

Diabetes was induced in mature (19 weeks old) male Sprague–Dawley rats by a single intraperitoneal (i.p.) injection of streptozotocin (40–45 mg/kg). The experimental design is illustrated in Fig. 1. After 4 weeks without treatment, during which NCV and blood flow deficits develop and stabilize (Cameron et al., 1989), groups of diabetic rats were given 4 weeks of treatment with IL-6 at one of 3 doses (1, 3 and 10 μg/kg s.c. 3 times per week). This treatment regime was found to give optimal results in a previous study (Andriambeloson et al., 2006). Untreated diabetic (4 weeks) and vehicle-treated (sterile saline with 0.02% rat serum albumin) nondiabetic control and diabetic groups (8 weeks) were also employed.

Motor and sensory conduction velocity and sciatic nerve endoneurial perfusion

At the end of the treatment period, rats were anesthetized with thiobutabarbital (50–100 mg/kg i.p.). The trachea was cannulated for artificial ventilation and a carotid cannula was used to monitor mean systemic blood pressure. Motor conduction velocity was measured between sciatic notch and knee in the nerve branch to tibialis anterior muscle, which is representative of the whole sciatic nerve in terms of susceptibility to diabetes and treatment effects (Cameron et al., 1989). Saphenous sensory conduction velocity was measured between groin and ankle as previously described (Cameron et al., 1989). Rectal and nerve temperatures were monitored and regulated between 36.5 and 37.5 °C.

Sciatic endoneurial blood flow was measured in the contralateral leg using microelectrode polarography and H_2 clearance as previously described (Cameron et al., 1991). Briefly, core temperature of the rat was monitored and regulated between 37 and 38 °C, using a rectal probe and radiant heat. The skin around the sciatic nerve incision was sutured to a metal ring and used to form a pool, which was filled with mineral oil. Pool temperature was maintained between 35 and 37 °C by radiant heat. Rats were artificially ventilated. The level of anesthesia was monitored by observing any reaction of blood pressure to manipulation, and supplementary anesthetic was given as necessary. A glass-insulated H_2-sensitive platinum polarographic microelectrode was inserted into the middle portion of the sciatic nerve, above its trifurcation. 10% H_2 was substituted for N_2 in the inspired gas. When the electrode H_2 current had stabilized, indicating equilibrium with arterial blood, the H_2 supply was shut off and the clearance curve recorded until baseline. This procedure was then repeated at another nerve site. After the experiment, clearance curves were digitized and mono-exponential or bi-exponential curves were fitted to the data by computer using non-linear regression software that employed the Marquardt algorithm and the least squares method for optimizing goodness-of-fit (Prism, Graphpad, San Diego, CA, USA). The slow exponent was taken to reflect nutritive (capillary)

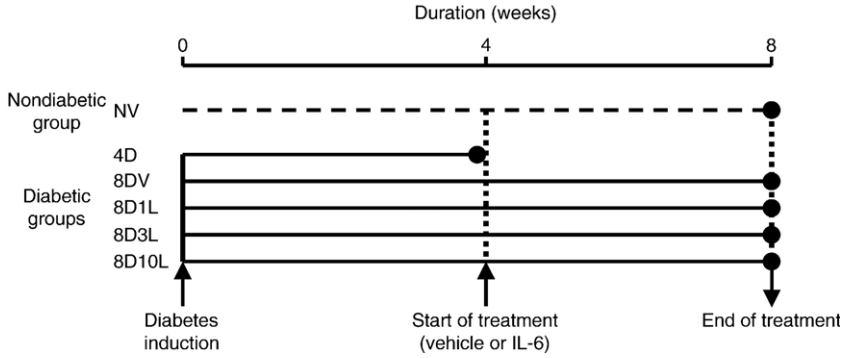

Fig. 1. Experimental design of the 8-week study. After diabetes induction by streptozotocin, rats were untreated for 4 weeks, before measurements were made to establish starting diabetic values, or were treated with vehicle or IL-6 for a further 4 weeks. Times of final measurements on nerve conduction, sensory tests and blood flow are denoted by filled circles. NV, vehicle-treated nondiabetic control group; 4D, 4-week diabetic control group; 8DV, 8-week diabetic group treated with vehicle for the last 4 weeks; 8D1L, 8D3L, 8D10L, 8-week diabetic groups, treated with IL-6 for the last 4 weeks at doses of 1, 3 and 10 μg/kg thrice weekly, respectively.