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Neuroprotective effects of chronic administration of levetiracetam in a rat model of diabetic neuropathy

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ABSTRACT

Objective: Diabetic neuropathy (DNP) is a frequent and serious complication of diabetes mellitus (DM) that leads to progressive and length-dependent loss of peripheral nerve axons. The purpose of the present study is to assess the neuroprotective effects of levetiracetam (LEV) on DNP in a streptozotocin (STZ)-induced DM model in rats.
Methods: Adult Sprague-Dawley rats were administered with STZ (60 mg/kg) to induce diabetes. DNP was confirmed by electromyography (EMG) and motor function test on 21st day following STZ injection. Study groups were assigned as follows; Group 1: Naïve control ($n = 8$), Group 2: DM + 1 mL/kg saline ($n = 12$), Group 3: DM + 300 mg/kg LEV ($n = 10$), Group 4: DM + 600 mg/kg LEV ($n = 10$). LEV was administered i.p. for 30 consecutive days. Then, EMG, motor function test, biochemical analysis (plasma lipid peroxides and total anti-oxidant capacity), histological and immunohistochemical analysis of sciatic nerves (TUNEL assay, bax, caspase 3, caspase 8 and NGF) were performed to evaluate the efficacy of LEV.
Results: Treatment of diabetic rats with LEV significantly attenuated the inflammation and fibrosis in sciatic nerves and prevented electrophysiological alterations. Immunohistochemistry of sciatic nerves showed a considerable increase in bax, caspase 3 and caspase 8 and a decrease in NGF expression in saline-treated rats whereas LEV significantly suppressed apoptosis markers and prevented the reduction in NGF expression. Besides, LEV considerably reduced plasma lipid peroxides and increased total anti-oxidant capacity in diabetic rats.
Conclusions: The results of the present study suggest that LEV may have therapeutic effects in DNP through modulation of anti-oxidant and anti-apoptotic pathways.

1. Introduction

Diabetic neuropathy (DNP) is the most common complication of diabetes mellitus (DM), which occurs in more than 50% of patients and affects nerve fibers of peripheral nervous system. The patients often present with loss of feeling and numbness in their feet, hands, and legs, which may be accompanied by excessive sensitivity to nociceptive stimuli or may perceive normal stimuli as painful [1–3]. To date, numerous

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mechanisms have been proposed to explain the relationship between the severity of hyperglycemia and the development of DNP including increased polyol pathway activity which leads to accumulation of sorbitol and fructose, reduction in Na^+K^+–ATPase activity, abnormal protein kinase C (PKC) activity, formation of advanced glycation end-products and auto-oxidation of glucose leading to the generation of reactive oxygen species [1–4]. In addition, metabolic dysfunction is accompanied by vascular deficiency and nerve hypoxia, which may contribute to nerve fiber loss and injury in diabetes. Based on these studies, various therapeutic agents including aldose reductase inhibitors (ARIs), anti-oxidants, selective PKC inhibitors, and neurotrophic factors have been used to improve peripheral nerve dysfunction in diabetic animals and patients [5–8].

Levetiracetam (LEV), an analogue of the nootropic agent piracetam, is an effective antiepileptic drug. Although the molecular effects of LEV remain uncharacterized, various studies indicate that it regulates the influx of calcium into the cells, selectively blocking N-type, but not the T-type channel [9–11]. Besides, it modulates membrane depolarization and prevents irreversible cellular damage *via* reducing the flow of potassium within the cell [12]. It has been reported that it binds synaptic vesicle protein (SV2A) and produce neuroprotective effects *via* modulating SV2A function [13]. On the other hand, LEV may have a direct ability to protect cells against kainic acid-induced toxicity *via* inhibition of lipid peroxidation [14].

To date, numerous clinical and experimental studies have suggested the protective effects of LEV on hypoxic ischemic brain injury, traumatic brain injury, subarachnoid hemorrhage and post-stroke epilepsy [15–17]. However, there is still limited data concerning its potential neuroprotective properties against peripheral neuropathies, such as DNP. In the present study, we hypothesized that utilization of different doses of LEV might have beneficial effects on peripheral nerve damage in diabetes. To accomplish this, we tested the effects of low and high doses of LEV on the functional and architectural properties of the sciatic nerve in Type I diabetic rats using electrophysiological, histological, immunohistochemical and biochemical parameters.

2. Materials and methods

2.1. Animals

Forty-two adult male Sprague Dawley rats weighing 210–230 g (221.4 ± 1.89 g) at the beginning of the experiments were used. The rats were housed in cages and maintained under standard conditions with 12-h light/dark cycles at room temperature (22 ± 2 °C). They were fed by standard pellet diet and tap water *ad libitum* throughout the study. All animal care and experimental procedures were approved by the Institutional Animal Care and Ethical Committee. All chemicals were obtained from Sigma-Aldrich Inc. unless otherwise noted.

2.2. Study design

Diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ; 60 mg/kg, Sigma-Aldrich Inc., St. Louis, MO) following an overnight fast. STZ was prepared in 0.9% NaCl and adjusted to a pH 4.5 with citric acid [18]. Diabetic state was verified 48 h later by determining tail vein blood glucose levels by glucose oxidase reagent strips (Boehringer-Mannheim, Indianapolis, IN). Animals showing blood glucose levels above 250 mg/dL were included in the study. Eight rats served as naïve control group and received no treatment. Control and diabetic rats were housed in their cages for 20 days. On day 21, EMG recordings were performed from the sciatic nerve under ketamine/xylazine anesthesia to confirm DNP. Following EMG studies on day 21, rats were divided into 4 groups. Group 1: Naïve control (n = 8), Group 2: DM + 1 mL/kg saline (n = 12), Group 3: DM + 300 mg/kg LEV (n = 10), Group 4: DM + 600 mg/kg LEV (n = 10). LEV (Keppra Flakon, UCB Farma) was diluted in saline and administered i.p. for 30 consecutive days. The doses were selected based on previous studies [19,20]. Following these treatments, gross motor test and EMG were performed. Blood samples were collected for biochemical analysis, and then animals were perfused for histology, quantitative immunohistochemistry (bax, caspase-3, caspase-8, NGF) and TUNEL staining.

2.3. Electrophysiological recordings (EMG)

Rats were anesthetized by combination of ketamine hydrochloride at a dose of 40 mg/kg (Alfamine®, Alfasan International B.V. Holland) and 4 mg/kg of xylazine hydrochloric (Alfazyne®, Alfasan International B.V. Holland). EMG was obtained for three times at the same time point from the right sciatic nerve stimulated supra-maximally (intensity 10 V, duration 0.05 ms, frequency 1 Hz, in the range of 0.5–5000 Hz, 40 kHz/s with a sampling rate) by a Biopac bipolar subcutaneous needle stimulation electrode (BIOPAC Systems, Inc., Santa Barbara, CA) from the Achilles tendon. Compound muscle action potentials (CMAPs) were recorded from 2 to 3. Interosseous muscle by unipolar needle electrodes. Data were evaluated using Biopac Student Lab Pro version 3.6.7 software (BIOPAC Systems, Inc., Santa Barbara, CA), with distal latency, duration and amplitude of CMAP as the parameters. During the EMG recordings, rectal temperatures of the rats were monitored by a rectal probe (HP Viridia 24-C; Hewlett- Packard Company, Palo Alto, CA, USA) and the temperature of each rat was kept at approximately 36 °C to 37 °C by heating pad [20].

2.4. Assessment of gross motor function

The motor performances of the rats were evaluated by inclined-plate test according to the method described by Rivlin and Tator [21]. The device was an 18 × 18 cm^2 platform, which could be adjusted to provide a slope of varying degrees. Briefly, the rats were placed with their body axis perpendicular to the inclined plane. The initial angle of the inclined plate was 50 degrees. The incline angle slowly increased and the maximum angle of the plate on which the rat preserved its position for 5 s without falling was recorded as motor score. This procedure was repeated five times per