European Journal of Pharmacology 771 (2016) 162–172

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Neuropharmacology and analgesia

Effect of levetiracetam versus gabapentin on peripheral neuropathy and sciatic degeneration in streptozotocin-diabetic mice: Influence on spinal microglia and astrocytes

Heba M. Reda [a], Sawsan A. Zaitone [b,*], Yasser M. Moustafa [b]

[a] Medical Center, Port-Said University, Port-Said, Egypt
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
16 December 2015
Accepted 18 December 2015
Available online 19 December 2015

Keywords:
Gabapentin
Spinal glia
Hot plate test
Levetiracetam
Peripheral diabetic neuropathy
Von Frey test

ABSTRACT

Peripheral diabetic neuropathy develops in diabetic patients. The current study tested the antiallodynic and antihyperalgesic effects of the anticonvulsant drug, levetiracetam compared with the standard drug, gabapentin, in a model of streptozotocin-induced peripheral diabetic neuropathy. Male albino mice were injected intraperitoneally with streptozotocin (40 mg/kg) for five consecutive days to induce type 1 diabetes mellitus. After development of peripheral diabetic neuropathy, mice were then treated orally with 10 doses of levetiracetam or gabapentin (or vehicle). The effect of multiple doses of levetiracetam on the histopathology of sciatic nerve and spinal cord was tested. Furthermore, the effect of levetiracetam on the spinal expression of microglia and astrocytes was examined in comparison with gabapentin. Results indicated that the highest dose of levetiracetam and all doses of gabapentin increased the withdrawal threshold in von Frey test. Furthermore, all doses of levetiracetam and gabapentin prolonged the reaction time exhibited by diabetic mice tested in hot plate test. Both drugs provided protection for the sciatic nerve and the spinal cord. In addition, levetiracetam (20 and 40 mg/kg) decreased spinal immunostaining for $CD_{11}b$ (microglia marker) and glial fibrillary acidic protein (GFAP, astrocytes marker) however; the high dose of gabapentin (40 mg/kg) reduced the spinal immunostaining for GFAP only. In conclusion, levetiracetam produced antiallodynic and antihyperalgesic effect in diabetic mice with favorable effects on sciatic nerve and spinal cord that were accompanied by downregulation of the spinal expression of microglia and astrocytes. Thus, levetiracetam may have promise in alleviating neuropathic pain in diabetic patients.

1. Introduction

Painful peripheral diabetic neuropathy is a significant cause of pain and distress in diabetic patients with diabetes mellitus (Backonja and Serra, 2004). There are several manifestations that are observed in peripheral diabetic neuropathy like thermal hyperalgesia, mechanical hyperalgesia and tactile allodynia (Obrosova, 2009). Previous studies highlighted that hyperalgesia can develop in animal models of streptozotocin (STZ)-induced diabetes mellitus (Khan et al., 2008, Sood et al., 2000). Peripheral diabetic neuropathy is a type of neuropathic pain associated with progressive degeneration of nerve fibers due to hyperglycemia (Boulton et al., 2005). The symptoms appear first in the longest axons with pain starting in the feet followed by distal lower extremities and finally affects the hands (Galer et al., 2000).

The role of spinal glia cells in the development of diabetic neuropathy has been appreciated (Daulhac et al., 2006). Spinal glial cells include microglia which represent 5–10% of glia in the central nervous system (Nakajima and Kohsaka, 2001). They are often considered as resident macrophages. They act as sensors for a range of stimuli that threaten physiological homeostasis. Once activated, microglia showed a progressive series of changes in morphology, gene expression, function and number (Inoue et al., 1997). They express a variety of cell-surface molecules including cluster determinant$_{11}$b ($CD_{11}b$), which is usually recognized by the anti-$CD_{11}b$ (Hickman et al., 2008). Astrocytes are the most abundant macroglia cells in the CNS (Mika et al., 2013). Activation of astrocytes leads to morphological changes, such as hypertrophy and increased production of glial fibrillary acidic protein (GFAP) (Zhang et al., 2005); astrocytes marker which can be recognized by anti-GFAP (Liesi et al., 1983) and functionally increased production of diverse molecules, including pro- inflammatory cytokines (Jang et al., 2013; Sun et al., 2008; Watkins and Maier, 2003).

There are many medications to relieve pain associated with

* Corresponding author.
E-mail address: Sawsan_zaytoon@pharm.suez.edu.eg (S.A. Zaitone).

http://dx.doi.org/10.1016/j.ejphar.2015.12.035
0014-2999/

peripheral nerve damage. Many clinical studies indicated efficacy of gabapentin on neuropathic pain in postherpetic neuralgia (Field et al., 1997) with FDA approval, diabetic neuropathy (Chou et al., 2009) and spinal cord injury (Tzellos et al., 2008). Levetiracetam is an anticonvulsant drug that binds to a synaptic vesicle glycoprotein 2A (Lynch et al., 2004), inhibits presynaptic calcium channels (Vogl et al., 2012) and impedes impulse conduction across synapses (Rogawski, 2006). Some animal studies demonstrated the efficacy of levetiracetam in treating peripheral neuropathic pain associated with diabetes (Ardid et al., 2003; Ozcan et al., 2008).

The present study tested the ameliorative effect of repeated treatment with levetiracetam on diabetic neuropathy in a model of STZ-induced diabetes, compared with a standard drug, gabapentin. Since the peripheral diabetic neuropathy can affect central and peripheral nerves, the present study evaluated the effect of the treatment with levetiracetam on the histopathological changes of spinal cord and sciatic nerve, compared with gabapentin. Further, spinal glia is critical in the pathology of peripheral diabetic neuropathy. Hence, the current study examined the involvement of these glial cells in the antiallodynic and antihyperalgesic effects of both drugs.

2. Materials and methods

2.1. Animals

Healthy male Swiss albino mice, weighing 19–31 g were used for the experiments. Mice were provided by the modern veterinary office for laboratory mice (Cairo, Egypt) and were housed in clean cages with food and water *ad libitum*. Mice were maintained under constant laboratory conditions and a normal light–dark cycle and were allowed to acclimatize for one week before use. All efforts were made to minimize crowding or mouse suffering during handling or injection. The experimental protocol was approved by the research ethics committee at the Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt. All experiments were carried out between 11:00 and 15:00 h to avoid the circadian influence on mice behavior.

2.2. Chemicals and drugs

Streptozotocin was purchased from Sigma-Aldrich (St Louis, Missouri, USA) and was dissolved in citrate buffer at pH=4.5. Levetiracetam was kindly provided by Multi-Apex Company (Cairo, Egypt) and gabapentin was obtained from Delta Pharma Company (El-Asher Men-Ramadan City, Egypt); they were dissolved in distilled water.

2.3. Induction of type 1 diabetes mellitus in mice

Following an overnight fast, experimental type 1 diabetes mellitus was induced in mice by five low doses of freshly prepared STZ (40 mg/kg/day, i.p.) over five consecutive days (Leiter, 1982; Wiggin et al., 2008). In general, STZ or the vehicle (citrate buffer) were injected daily in a volume equals 10 ml/kg (i.p.) for five days. This technique was used to mitigate nonspecific cytotoxicity resulting in repetitive low grade cell damage of pancreatic islet cells (Like et al., 1987). Hyperglycemia was confirmed within 1 week by measuring the fasting blood glucose level using a blood sample from the tail vein with the aid of an Accu-Check glucometer. Mice with final blood glucose levels >250 mg/dl were considered diabetic and included in the study (Anjaneyulu and Ramarao, 2002, Ortiz-Ramirez, 2012).

2.4. Experimental design

Six weeks after confirming the development of hyperglycemia, diabetic mice were tested for peripheral diabetic neuropathy (Goss et al., 2002) using von Frey filaments (a test for tactile allodynia) and hot plate test at 55 °C (a test for thermal hyperalgesia). Diabetic mice showing preliminary thermal hyperalgesia were allocated into different groups, 8 mice in each group. Mice were subjected to ten therapeutic doses of levetiracetam or gabapentin.

Briefly, mice were divided into eight different groups. [1] vehicle (citrate buffer), [2] diabetic control, [3–5] diabetic+levetiracetam (10, 20 or 40 mg/kg, p.o.), respectively (AL-Baker, 2008), [6–8] diabetic+gabapentin (10, 20 or 40 mg/kg, p.o.), respectively (Yasuda et al., 2005). In general, oral treatment with levetiracetam or gabapentin (12 ml/kg, p.o) was initiated at the beginning of week 8, every 48 h, for a total of 10 doses. Diabetic control mice received 10 doses of distilled water (12 ml/kg, p.o.) at the same schedule reported for drug therapies using an oral tube. At the last day of week 10, the final assessment of pain behavior using von Frey filaments and hot plate test was done and the ataxic effect was determined using the rotarod test.

2.5. Justification of doses of levetiracetam and gabapentin

In the present study, levetiracetam (10, 20 and 40 mg/kg) was used for treatment of diabetic neuropathy. These doses can be translated to the human equivalent doses by using the Reagan-Shaw method (2008). Upon applying this formula, the human equivalent dose (mg/kg)=animal dose (mg/kg) × animal (km)/human (km). km for an adult human (60 kg) equals 37 and for a mouse equals 3. Therefore, the dose equivalent to a mouse dose of 10–40 mg/kg will be approximately 50–200 mg for an adult human with body weight equals 60 kg. The typical human dose of levetiracetam is 1000 mg/day, given as twice-daily dosing (500 mg twice daily) and dose increments are common up to 3000 mg/day. For gabapentin, it is commonly given orally for patients with neuropathy at 900–1800 mg/day divided at 3 doses. Therefore, all the selected doses in the present study are within the safe therapeutic range recorded in humans.

2.6. Von Frey filaments (tactile allodynia)

The right hind paw withdrawal threshold in response to a normally innocuous mechanical stimulus was reported using von Frey filaments and the up–down method (Chaplan et al., 1994). Mice were placed individually in a plastic cage with a wire mesh bottom and allowed to acclimatize to this environment for 30 min before testing. Mechanical threshold was determined through applying a series of von Frey filaments (0.16, 0.6, 1.4, 4, 10, 60, 100, 180 and 300 g) in ascending forces. The filaments were pressed perpendicular to the median plantar surface of the right hind paw of each mouse. Prolonged hind paw withdrawal, licking or biting of the hind paw was considered positive responses. Each filament was tested for 5 times per paw and the mechanical threshold was defined as “the minimal force that caused at least 3 withdrawals observed out of 5 consecutive trials” (Yalcin et al., 2009).

2.7. Hot plate test (thermal hyperalgesia)

The hot plate test is a test for the pain response in mice after exposure to an acute noxious thermal stimulus. It is usually employed to measure the response latencies as described previously by (Eddy and Leimbach, 1953). The hot-plate apparatus (Lsi LETICA, model LE 7406, Italy) consists of a transparent glass cylinder of 20-cm diameter and 25-cm height, digital set point, a built in electronic timer and foot switch timing operation. The