European Journal of Pharmacology 769 (2015) 297–305

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural pharmacology

Favorable effects of vildagliptin on metabolic and cognitive dysfunctions in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article history:
Received 15 August 2015
Received in revised form
16 November 2015
Accepted 18 November 2015
Available online 21 November 2015

Keywords:
Vildagliptin
Diabetes mellitus
Dipeptidyl peptidase-IV
Cognition
Streptozotocin

Chemical compounds studied in this article:
Vildagliptin (PubChem CID: 11077541)
Streptozotocin (PubChem CID: 2733335)

ABSTRACT

Progression of diabetes mellitus is accompanied by metabolic disorders together with psychological deficits including cognitive dysfunctions. Herein, we used a murine streptozotocin (STZ)-induced diabetes to investigate the beneficial effects of vildagliptin not only on metabolic abnormalities, but also on diabetes-induced cognitive decline. Sixty rats were divided randomly and equally into 2 groups; one remains normal and the other serves as STZ- induced diabetic. Both groups were further divided equally into 2 groups; one received vehicle and the other received oral vildagliptin for 8 weeks. Cognitive behavior was assessed using novel object recognition test. Blood samples were collected to measure metabolic parameters and dipeptidyl peptidase (DPP)-IV activity. Brains were removed and investigated for the levels of inflammatory and oxidative stress markers malondialdehyde (MDA), superoxide dismutase (SOD) and tumor necrosis factor-α (TNF-α), in addition to brain-derived neurotrophic factor (BDNF) and relative expression of nuclear factor kappa B (NF-κB)/p65. Treatment of STZ-induced diabetic rats with vildagliptin increased their body weight and corrected diabetes-induced memory and learning impairment. Moreover, vildagliptin significantly decreased serum levels of glucose and lipids (except high density lipoprotein) together with brain MDA, TNF-α, serum DPP-IV activities and NF-κB/p65 gene expression. On the other hand, vildagliptin significantly increased brain BDNF, SOD as well as serum insulin. Results suggested that vildagliptin has a protective role in counteracting both metabolic abnormalities and memory deficits in diabetic rats, possibly via its anti-hyperglycemic, anti-inflammatory, antioxidant effects, together with reduction of brain NF-κB/p65 over expression.

1. Introduction

Diabetes mellitus (DM) is one of the most common endocrinal metabolic diseases with multi-organ affection and heterogeneous complications, primarily due to hyperglycemia. Several studies underlined the central nervous system complications of DM and many research efforts were dedicated to demonstrate its association with the under-investigated pathophysiological changes in the brain that result in increased risk for cognitive dysfunctions with varying degrees (Kodl and Seaquist, 2008). Diabetes-associated cognitive decline (DACD) is a form of the diabetes-associated long-term end-organs damage that could be attributed to several factors including, but not limited to, poor glycemic control (Perantie et al., 2008) and brain-inflammatory and oxidative stress-induced cellular and molecular damage (Martin-Gallan et al., 2007) during DM progression. For proper management of the DACD, it is important to delineate its pathogenesis to further explore the potential preventive and therapeutic approaches for such deficits (Biessels et al., 2014).

The crucial role of hyperglycemia in the generation of cognitive decline in diabetic patients implies that drugs which improve glycemic control may be useful for ameliorating DACD. The role of intestinal hormones called incretins, including glucagon-like peptide-1 (GLP-1) (Proglucagon (72–108) in the regulation of glucose homeostasis has been explored as new remedies for DM with novel mechanisms of action (Baggio and Drucker, 2007). Glucagon-like peptide-1 was shown to have an important role in cognitive

Abbreviations:: (AD), Alzheimer's disease; (BDNF), brain-derived neurotrophic factor; (CNS), central nervous system; (DACD), diabetes-associated cognitive decline; (DM), diabetes mellitus; (DPP)-IV, dipeptidyl peptidase; (ELISA), enzyme-linked immunosorbent assay; (EDTA), ethylene diamine tetra acetic acid; (HDL-C), high density lipoprotein-cholesterol; (ILAR), Institute of Laboratory Animal Resources; (IL-1β), interleukin-1β; (LDL-C), low density lipoprotein-cholesterol; (MDA), malondialdehyde; (NBT), nitro-blue tetrazolium; (NOR), novel object recognition; (NF-κB), nuclear factor kappa B; (ROS), reactive oxygen species; (RT-PCR), real-time polymerase chain reaction; (RI), recognition index; (STZ), streptozotocin; (SOD), superoxide dismutase; (TC), total cholesterol; (TAG), triacylglycerol; (TNF-α), tumor necrosis factor-α

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http://dx.doi.org/10.1016/j.ejphar.2015.11.033
0014-2999/

functions integrity and attenuated deficits in memory and behavior (Chen et al., 2012). However, endogenous GLP-1 is rapidly inactivated by a membrane and soluble protein identified in 1993, namely; Dipeptidyl peptidase-IV (DPP-IV) (Lambeir et al., 2003).

The very short $t_{1/2}$ of GLP-1 mandated researchers to investigate the antidiabetic potential of inhibiting DPP-IV to prolong the active glucagon-like peptide-1 duration of action that enhanced the physiological insulin secretion (Richter et al., 2008). Recently, research efforts revealed that DPP-IV inhibitors can be used as antidiabetic agents to allow proper glycemic control without high risk of hypoglycemia by preventing the rapid degradation of GLP-1 (Singh, 2014).

Vildagliptin is a selective competitive reversible inhibitor for DPP-IV. It is safe, with low risk of hypoglycemia, less interactions and neutral effect on weight (Vella et al., 2007). Proposed mechanisms of vildagliptin-glucose controlling actions are mediated mainly by increasing availability of circulating GLP-1 with subsequent improved pancreatic β-cells functions, which in turn increase insulin secretion in response to food, and decrease hepatic glucose output by decreased glucagon secretion, decrease insulin resistance and restore pancreatic β-cells mass (Ahren et al., 2011).

Herein, for the first time in rats with streptozotocin (STZ)-induced DM, the effects of vildagliptin not only on the associated metabolic abnormalities, but also on cognitive deficits are investigated, in addition to delineating the underlying neuroprotective mechanisms.

2. Materials and methods

2.1. Chemicals and drugs

Most chemicals, including streptozotocin, were purchased from Sigma Aldrich Chemical Co., St. Louis, MO, USA, stored at 2–4 °C and protected from sunlight. Vildagliptin (Novartis) is a DPP-IV which was commercially acquired and was diluted in phosphate buffer suspended in 0.05% methylcellulose in a final concentration of 5 mg/kg body weight/day (Avila et al., 2013). The glucose oxidase diagnostic enzyme, lipid profile kits were obtained from Span Diagnostic Chemicals. Enzyme linked immunosorbant assay (ELISA) kits for insulin, tumor necrosis factor alpha (TNF-α) and brain derived neurotrophic factor (BDNF) were purchased from R & D Systems (USA). All chemicals used for biochemical estimations were of analytical grade.

2.2. Animals and experimental model

Male adult Sprague Dawley rats weighing 200–240 g were allowed to acclimatize for 7 days. A single intraperitoneal injection of 65 mg/kg STZ in freshly prepared 0.1 M citrate buffer (pH 4.5) to induce type 1 diabetes in rats (Tuzcu and Baydas, 2006). Three days after STZ injection, diabetes was confirmed using a digital glucometer (Bionime Rightest GM100; Taiwan) on blood droplets obtained from the rats' tail veins. The animals that showed fasting blood glucose higher than 250 mg/dl were included in the study (Kuhad et al., 2008). Rats were randomly assigned into 2 equal groups; diabetic vildagliptin-treated (group DV) received vildagliptin 5 mg/kg per day by gavage for eight weeks starting from the third day of experiment (after diabetes was induced), and diabetic-control untreated (group D) which received vehicle (Ola et al., 2014). On the other hand, age, weight and strain matched naïve rats received an intra-peritoneal equal volume of citrate buffer and were randomly assigned into 2 equal groups; control untreated (group C) and control vildagliptin-treated (group CV). Body weight and blood glucose levels were determined before and at the end of the experiments. At the beginning of the experiment, animals had similar glucose levels. After treatment for 8 weeks, the animals were not tested for learning and memory task by novel object recognition (NOR) test. The next day after NOR task the rats were fasted overnight, and then killed by cervical decapitation. After decapitation, the blood samples were collected, and brains were rapidly removed. The samples were stored at −80 °C until processed for biochemical measurements.

During the study, animals were housed in wire mesh cages and were fed standard rat chew and allowed free access to water. They were kept under controlled environment, at a constant temperature (23 ± 2 °C), humidity (60 ± 10%) and light/dark (12/12 h.) cycle. Every effort was made to avoid and minimize stress to the animals. All experiments were conformed to Guidelines for Ethical Conduct in the Care and Use of Animals. Animal handling and experimental protocols were approved by the Ethical Committee of the Faculty of Medicine, Menoufia University, Egypt, and comply with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

2.3. Nor tasks

The novel object recognition test measures the ability of rats to recognize a novel object in an otherwise familiar environment. In the NOR test, the interest of an animal in a novel object versus a familiar one is measured and compared. If the exploration of the novel and the familiar object is equal, this can be interpreted as a memory deficit (Dix and Aggleton, 1999). The object recognition task was carried out in a 40 × 60 × 50 cm^3 Plexiglas open field. The floor of the open field was divided into 12 equal rectangles by black lines. The rats of all groups were submitted to a habituation session where they were allowed to freely explore the open field for 5 min. No objects were placed in the box during the habituation trial. During open field (5 min), motor activity (distance measured by the number of crossed lines in the arena), exploration (number of rearing events) and anxiety (number of grooming events) were recorded. Twenty-four hour after habituation, familiarization trial was conducted by placing the individual rats in the open field for 5 min in which two identical objects (objects A1 and A2; both being cubes) were positioned in two adjacent corners 10 cm away from the walls. Four h later, the test trial was conducted by exposing the animals to the familiar object and a novel object placed at the same locations for 5 min, during which, the time exploring each object was recorded. A minimum of 10 s of exploration were required, a criterion met by all rats in these experiments (Fig. 1).

2.4. Blood and serum analyses

Blood was collected from the four groups of animals into dry sterile centrifuge tubes, centrifuged for 10 min at 4000 × *g*. Serum was separated and stored at −80 °C for further analysis of the following:

1. Glucose blood level was measured by the glucose oxidase method using a commercial kit (Elitech Diagnostics Company, France) according to the manufacturer's instructions.
2. Total cholesterol (TC), triacyglycerols (TAG) and high density lipoprotein cholesterol (HDL-C) levels were measured using the appropriate kits (Boehringer Mannheim, Germany) according to the manufacturer's instructions. Serum low density lipoprotein cholesterol (LDL-C) values were calculated using the Friedewald formula as follows: LDL-C = TC − (HDL-C + TAG/5).
3. Insulin serum level was measured by ELISA using commercial kit purchased from R & D Systems (USA) according to (Matveyenko et al., 2008).
4. Serum Dipeptidyl peptidase-IV activity was determined with