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Sitagliptin attenuates transient cerebral ischemia/reperfusion injury in diabetic rats: Implication of the oxidative–inflammatory–apoptotic pathway

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ABSTRACT

Aims: Ischemic stroke is a major macrovascular complication of diabetes mellitus. Sitagliptin, a dipeptidyl peptidase-IV inhibitor, was recently shown to improve cognitive functions in diabetic rats; hence the present study was conducted to evaluate its protective effect against transient ischemia–reperfusion (I/R) in diabetic animals.

Main methods: Diabetes was induced by streptozotocin (40 mg/kg). Six weeks later, cerebral I/R was induced by bicommon carotid occlusion for 15 min followed by 1 h reperfusion. Sitagliptin (250 mg/kg; p.o.) was administered daily during the last 2 weeks before I/R.

Key findings: The drug alleviated hippocampal injury inflicted by diabetes and/or I/R injury where it suppressed nuclear factor kappa (NF-κ)B, and consequently the downstream inflammatory cytokines tumor necrosis factor-α and interleukin-6. In parallel, the anti-inflammatory cytokine interleukin-10 was elevated. Antioxidant potential of sitagliptin was depicted, where it reduced neutrophil infiltration, lipid peroxides and nitric oxide associated with replenished reduced glutathione. Decline of excitatory amino acid glutamate content is a main finding which is probably mediated by the NF-κB signaling pathway as well as improved oxidant status. Sitagliptin exerted an anti-apoptotic effect as reflected by the reduction of the mitochondrial matrix component cytochrome -C and the key downstream executioner caspase-3. Histopathological examination corroborated the biochemical data.

Significance: These findings suggest that sitagliptin is endowed with neuroprotective properties which are probably mediated by its antioxidant, anti-inflammatory, and anti-apoptotic mechanisms and hence may provide a novel agent for the management of ischemic stroke in diabetics.

Introduction

Stroke is the second leading cause of death worldwide [13]. Epidemiological studies provide evidence that diabetes exacerbates and/or is a principal risk factor of stroke [55]. In fact, diabetic patients are two to four times more predisposed to go through a stroke than non-diabetics. They consistently exhibit poor functional outcomes and prognoses, and are more prone to morbidity and mortality after stroke [24]. Diabetics are also more vulnerable to stroke recurrence [26].

The mechanisms by which diabetes aggravates ischemic brain damage are not yet fully understood. However, vascular physiological and morphological alterations are prominent in diabetes and presumably contribute to the marked damage [57]. Besides, elevated blood glucose level stimulates pro-inflammatory cytokines, promotes lipid peroxidation, and further activates the apoptotic pathway which altogether contribute to the pathophysiology of various diabetic complications [12]. Accordingly, long-term maintenance of normal or near-normal glucose level using pharmacological agents improves macrovascular complications and reduces morbidity and mortality in diabetic patients [5].

Gliptins have recently emerged as effective and well tolerated oral antidiabetic agents for the management of non insulin-dependent diabetes mellitus. They increase incretin content due to inhibition of dipeptidyl peptidase-IV (DPP-IV) activity, which is responsible for the breakdown of the glucagon like peptide-1 (GLP-1) hence greatly affecting both insulin and glucagon secretions [54]. Interestingly, it was reported that GLP-1R is expressed throughout the brain, including the hippocampus [20]. GLP-1 produced in the gut not only crosses the blood–brain barrier, but the peptide is also synthesized in the brain exerting central anti-inflammatory and antiapoptotic effects thus impeding neuronal damage [35].

While the hypoglycemic effects of DPP-IV inhibitors have been widely investigated in both experimental and clinical studies, their outcome on cerebral ischemia is poorly ascribed. Thus the present study aimed to evaluate whether sitagliptin, the first gliptin to be clinically

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introduced, can amend the deleterious effects of cerebral ischemia in diabetic rats. Recently, it was demonstrated that the drug ameliorated myocardial [21] as well as renal [8] infarctions in rats. Moreover, it was reported that sitagliptin possesses anti-oxidant and anti-inflammatory properties [31] which raises the possibility of its beneficial role to ameliorate neuronal damage associated with ischemic stroke.

Inevitably, cerebral ischemia creates an inflammatory milieu in the affected brain regions in the settings of microglia and astrocyte activation. Inflammatory reactions trigger free oxygen radical generation finally leading to apoptosis [19,39]. Hippocampus was reported as a susceptible region for neuroinflammation in ischemic stroke [41]. Therefore, the effects of sitagliptin on hippocampal oxidative stress and pro-inflammatory mediators, as well as apoptotic factors together with the excitatory neurotransmitter glutamate have been targeted herein.

Materials and methods

Chemicals

Streptozotocin (STZ), sitagliptin, and thiopental sodium were purchased from Sigma (St. Louis, MO, USA). Sitagliptin was dissolved in 1% Tween 80. All other chemicals were of the highest purity and analytical grade.

Animals

Male Wistar albino rats weighing (200–250 g) were used in the present study. They were allowed an acclimatization period for at least one week prior to testing. Animals were kept under controlled environmental conditions; room temperature (24–27 °C), constant humidity (60 ± 10%), with alternating 12 h light and dark cycles. Food (standard pellet diet) and water were allowed ad libitum. All animals' procedures were performed in accordance with the ethical procedures and policies approved by the Ethics Committee of the Faculty of Pharmacy, Cairo University and in accordance with the recommendations for the proper care and use of laboratory animals.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of STZ (40 mg/kg), freshly prepared in 0.1 M citrate buffer, pH 4.5 [38]. Rats were allowed to drink 5% glucose solution during the first 24 h of diabetes induction to overcome the drug-induced hypoglycemia [34]. Two days later, blood samples were collected from rats' tails and hyperglycemia was confirmed by a blood glucose level reaching 300 mg/dl. Glucose was measured using an analyzer (Roche Diagnostic Accu-Check Test Strips, Germany).

Induction of transient cerebral ischemia

Rats were anesthetized with thiopental (50 mg/kg, i.p.) and a midline ventral incision was made in the neck. Bilateral carotid artery occlusion using small artery clips was used to induce global cerebral ischemia for 15 min followed by a 60 min reperfusion period [1].

Experimental design

One hundred rats were randomly allocated into 5 groups (20 rats each). Groups I and II were normal healthy rats that received a single intraperitoneal injection of citrate buffer and were kept for a six week period. Group I served as normal sham operated control, while group II rats received 1% Tween 80 (10 ml/kg; p.o.) daily during the 5th and 6th weeks after which transient I/R was induced and served as the I/R control group.

Groups III, IV and V were diabetic rats that received a single intraperitoneal injection of STZ dissolved in citrate buffer and were kept for a six week period. Group III served as diabetic sham operated group; group IV was subjected to I/R by the end of the 6th week and served as diabetic I/R control group, while Group V received sitagliptin (250 mg/kg; p.o. daily) [30] dissolved in 1% Tween 80 during the 5th and 6th weeks after which transient I/R was induced and served as the diabetic I/R sitagliptin treated group.

Immediately after reperfusion, animals in each group were sacrificed by cervical dislocation and both hippocampi of each rat were isolated. Each group was further subdivided into three sets. The first set ($n = 6$) was used for ELISA estimations, the second one ($n = 6$) was employed for assessment of glutamate content by HPLC, while the last set ($n = 6$) served to determine the rest of the biochemical parameters. The remaining 2 brains were kept for histopathological examination.

Biochemical determinations in the hippocampus

Estimation of oxidative stress biomarkers

Lipid peroxide formation was determined in rat hippocampus homogenate (10% w/v normal saline) by estimating the content of thiobarbituric acid reactive substances (TBARS) using malondialdehyde (MDA) as a standard according to the method described by Mihara and Uchiyama [36]. Reduced glutathione (GSH) content was measured using Ellman's reagent as described by Beutler et al. [4]. Total nitrite and nitrate contents were assessed according to the method of Miranda et al. [37] based on the Griess reaction and employing vanadium trichloride as a reducing agent.

Estimation of inflammatory and apoptotic mediators

The kinetic method described by Bradley et al. [6] was employed to determine myeloperoxidase (MPO) activity. Since the enzyme is located within the primary granules of neutrophils, its extraction necessitates the disruption of the granules to render MPO soluble in aqueous solution. This was achieved by sonication in potassium phosphate buffer (50 mM, pH 6) containing 0.5% hexadecyltrimethylammonium bromide (HTAB), a detergent that releases MPO from the primary granules of the neutrophil [53].

Hippocampal contents of cleaved caspase-3, interleukin-6 (IL-6), interleukin-10 (IL-10), as well as tumor necrosis factor alpha (TNF-α) were assessed using enzyme-linked immunosorbent assay (ELISA) kits supplied by R&D Systems, Inc., Minneapolis, USA. Similarly, the contents of nuclear factor-kappa B (NF-κB) and cytosolic cytochrome-C were measured using ELISA kits supplied by EIAab Science Co., Wuhan, China.

Estimation of the excitatory amino acid glutamate

Glutamate content was estimated using a fully automated high-pressure liquid chromatography system (HPLC; Perkin-Elmer, MA, USA) according to the precolumn phenylisothiocyanate derivatization technique described by Heinrikson and Meredith [23]. Brain residues were reconstituted in a 2:2:1 mixture (v) of methanol:1 M sodium acetate trihydrate:triethylamine then re-dried under vacuum. The reaction of derivatization was performed for 20 min at room temperature using a 7:1:1:1 mixture (v) of methanol:triethylamine:double-distilled deionized water:phenylisothiocyanate, then subjected again to vacuum until dryness. Derivatized amino acids were reconstituted with a sample diluent consisting of a 5:95 mixture (v) of acetonitrile:5 mM phosphate buffer (pH = 7.2). After sonication, samples were filtered (0.45 μm; Millipore). A Pico-Tag physiological free amino acid analysis C18 (300 mm × 3.9 mm i.d.) column from Waters (MA, USA) and a binary gradient of Eluents 1 and 2 (Waters) were used. The mobile phase consisted of a mixture of a 0.1 M NaH_2PO_4 buffer, pH 6.2 containing 20% methanol (eluent 1) and a 0.01 M NaH_2PO_4 buffer, pH 5.8, 80% methanol (eluent 2). The column was eluted isocratically for 7 min with 95% eluent 1 and 5% eluent 2 followed by a gradient increasing the concentration of eluent 2 to 50% during 3 min at a flow rate of