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Effects of topiramate on diabetes mellitus induced by streptozotocin in rats

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ABSTRACT

Topiramate currently approved for marketing as antiepileptic drug also possesses anti-diabetic activity. The aim of this study was to determine the antidiabetic effect of topiramate in a rat model of diabetes mellitus. Diabetes was induced by a single injection of streptozotocin to fasted rats. Diabetic animals were divided into untreated; insulin treated; topiramate treated with 25, 50 and 100 mg/kg; and combined insulin plus topiramate treatment in the previous doses. All medications were given once daily started after the rise of blood glucose for three weeks. Control rats were divided into untreated; vehicle treated and rats given topiramate in the previous doses. Body weight, blood-glucose and insulin levels were measured. Histopathological examination, immunohistochemical and morphometric studies of islets of the pancreas were done. Topiramate 50 and 100 mg/kg resulted in a significant decrease in the blood glucose and increase in the insulin levels as well as the number of islets and the count and mass of beta cells. Combined treatment to diabetic rats with insulin and topiramate induced a better response than either alone. Further experimental and clinical studies are needed to explore the different mechanisms of action of topiramate as antidiabetic both in insulin dependent and non-insulin-dependent diabetes mellitus.

1. Introduction

The incidence and prevalence of diabetes mellitus have continued to increase globally, despite a great deal of research, with the resulting burden resting more heavily on tropical, developing countries. Diabetes mellitus is an endocrine disorder of carbohydrate metabolism resulting primarily from inadequate insulin release (type 1 insulin-dependent diabetes mellitus) or insulin insensitivity coupled with insufficient compensatory insulin release (type 2 non-insulin-dependent diabetes mellitus) (Wild et al., 2004).

Besides insulin, glucagon, and pancreatic polypeptides, islets secrete L-glutamate, gamma aminobutyric acid (GABA) and somatostatin as paracrine-like modulators (Hayashi et al., 2003; Kanno et al., 2002; Moriyama and Hayashi, 2003). Although the modes of action of these paracrine modulators are less characterized, they recently showed that α- and β-cells of the pancreas communicate with each other through L-glutamate and GABA, which act as intercellular transmitters, to regulate precisely their endocrine functions (Moriyama and Hayashi, 2003).

Topiramate is a novel therapeutic agent structurally unrelated to the other anticonvulsants, currently approved for marketing as an antiepileptic drug. It is a sulfamate-substituted monosaccharide, 2, 3:4, 5-bis-O-(1-methylethylid-ene)-beta-D-fructopyranose sulfamate; interestingly, topiramate was invented during a search for new antidiabetic drug (Sachdeo, 2003).

Although the precise mechanism of action of topiramate as antiepileptic drug is not known, studies have shown that topiramate seems to act through multiple mechanisms (Czapiński et al., 2005; Guerrini and Parmeggiani, 2006).

Clinical studies reported that topiramate treatment reduced body weight and decreased fasting blood glucose levels in obese patients with or without type 2 diabetes (Eliasson et al., 2007; Myung et al., 2009). It is unclear whether the blood-glucose-normalizing phenomenon observed during topiramate treatment is an independent primary effect or the consequence of reduced food intake and weight loss (Liang et al., 2005).

Based on the pharmacology, mechanisms and previous studies the present experiment was designed to demonstrate the blood glucose lowering effect of various doses of topiramate and whether this is through body weight lowering, insulin secretagogue or beta cell regenerating effects in streptozotocin induced diabetes mellitus in rats.

2. Material and methods

2.1. Experimental animals

This study was approved by our institution's (kasr el eini hospital) Animal Care Committee and the guidelines were strictly adhered to.

Male adult albino Sprague–Dawley rats, matched for age and weight (between 210 and 230 g), were used for the experiment. Rats

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were maintained on standard rat chow and tap water ad lib throughout the study; and were housed two per cage at room temperature.

2.2. Animal grouping

The animals were randomly assigned into two main groups; the number of rats in each group represented the final number after deaths.

Group 1 Diabetes was induced by a single intraperitoneal injection of freshly dissolved streptozotocin (STZ) 60 mg/kg body weight in 0.1 M sodium citrate buffer, pH = 4.5 (Ganda et al., 1976; Zahra et al., 2008), to 18 h fasted rats. STZ is diluted in recently prepared Na-Citrate buffer immediately prior to injection to avoid degradation of the STZ.
Animals with fasting blood glucose above 200 mg/dl were included in the study as diabetics (Mahmoud et al., 2009).
In a pilot study, measurement of fasting serum glucose concentration 48 h after injection of STZ identified diabetes with massive hyperglycemia. Blood glucose levels were maintained elevated for three weeks after induction of diabetes.

Topiramate solutions were prepared fresh by dissolving the powdered form of the drug in water.

Diabetic animals (48 rats) were randomly divided into four groups.

- Diabetic untreated rats (six rats).
- Insulin treated diabetic rats: diabetic rats were treated by insulin NPH (Insulin Zinc suspension) 17 unit/kg/day subcutaneously once per day in the morning (Haughton et al., 1999), started after rise of blood glucose, for three weeks (six rats).
- Topiramate treated diabetic rats: diabetic rats were treated by topiramate 25, 50 and 100 mg/kg (Jason et al., 2005; Kudin et al., 2004) in a volume of 3 ml water by oral gavage, started after the rise of blood glucose, once daily, for three weeks (18 rats).
- Insulin + topiramate treated diabetic rats: diabetic rats were treated by insulin s.c. plus 25, 50 and 100 mg/kg topiramate (oral) started after the rise of blood glucose, once daily, for three weeks (18 rats).

Death of some hyperglycemic animals was observed between days 2 and 4 post-STZ (60 mg/kg). Prior administration of 2-deoxy-D-glucose (2-DG), 0.5 ml intraperitoneally up to 30 min before STZ protects against its acute lethal β-cytotoxicity (Ganda et al., 1976).

Group 2 Control normoglycemic rats (30 rats): they were randomly divided into three groups.
- Untreated rats were left to the end of the experiment for histopathological examination of the normal pancreas (six rats).
- Normoglycemic rats were given equivalent volume of 0.1 M sodium citrate buffer (six rats).
- Normoglycemic rats were given topiramate 25, 50 and 100 mg/kg (oral) for the whole length of the experiment (18 rats).

2.3. Drugs, reagents and solutions

- Streptozotocin (STZ) powder: Sigma Chemical Company
- Topiramate solutions were prepared fresh by dissolving the powdered form of the drug in water: Ortho-McNeil-Janssen Pharmaceuticals, Inc.
- Insulin NPH (Insulin Zinc suspension): Novo Nordisk, Denmark
- Sodium citrate buffer: Fisher Scientific
- 2-Deoxy-D-glucose (2-DG): Sigma Chemical Company
- FLEX Polyclonal Guinea Pig Anti-Insulin kit: Life Trade Egypt Company under license of Dako Laboratories, Carpinteria, CA, USA

2.4. Measurements

2.4.1. Body weight

Body weight was measured in gram, at the start, 48 h, one week, two weeks and three weeks after STZ injection.

2.4.2. Biochemical parameter

Blood samples (about 1 ml) were obtained from the retro-orbital sinus of the fasted animals using heparinized capillary tubes.

Fresh samples were used for estimating fasting blood-glucose (mg/dl), using glucose enzymatic-colorimetric assay test (Diagnosticum Rt.). Samples were taken at the start of the experiment, 48 h, one week, two weeks and three weeks after STZ injection.

Samples for determination of fasting serum insulin (ng/ml) were taken at the start (basal), 48 h after diabetes induction and at the end of the experiments. Serum was stored at −20 °C and was analyzed using a Rat Insulin ELISA Kit (Crystal Chem Inc.).

2.4.3. Histological study

Animals were killed by cervical dislocation at the end of the experiment. The pancreas was fixed in Bouin's solution for 24 h, paraffin-processed. Paraffin-embedded sections were cut at 5 μm and stained with hematoxylin and eosin (HE), and subjected to light microscopic examination (Drury and Wallington, 1980).

2.4.4. Immunohistochemical and morphometric study of islets

Pancreatic section was mounted on separate slides coated with poly-L-lysine; sections were stained immunohistochemically with an indirect method using the labeled avidin–streptavidin method and guinea-pig anti-insulin serum. For all groups, negative controls were performed with substitution of the primary antibodies with phosphate-buffered saline (PBS) (Yavuz et al., 2003).

Morphometric analysis was made by the point-counting method using an 8 × 8 mm grid (256 squares and 289 intersections) mounted on the eyepiece of the microscope.

The islet profiles were examined to estimate (a) the number of islets in each section of the pancreas, (b) the beta cell mass (mg), and (c) the number of b-cells of the pancreatic islets. All sections were blinded before quantitation.

2.5. Statistical analysis

All values were expressed as mean ± S.D. obtained from a number of experiments (n). Computer package SPSS 9.0 was used for data management and analysis. ANOVA test was used for comparison between the groups in each study arm. Morphometric data were analyzed by the Student's t-test. Differences with $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Body weight

Prior to STZ administration, the average weight of the rats was 220 ± 10.5 g. The body weight of control rats treated with citrate and topiramate 25 mg/kg remained unchanged, while control rats under topiramate 50 and 100 mg/kg showed a decrease in the body weight that started after 7 days continued for 14 days and gradually reaching near normal weights by 21 days (Table 1).

The adult diabetic rats showed progressive significant weight loss that started 48 h after diabetes mellitus was experimentally induced