Micron 39 (2008) 1300–1305

Contents lists available at ScienceDirect

Micron

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

A quantitative study of sodium tungstate protective effect on pancreatic beta cells in streptozotocin-induced diabetic rats

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ARTICLE INFO

Article history:
Received 25 December 2007
Received in revised form 26 February 2008
Accepted 27 February 2008

Keywords:
Diabetes mellitus
Sodium tungstate
Stereology
Beta cell

ABSTRACT

Diabetes is a major public health problem. Development of new therapies that are able to improve glycemia management, cure diabetes, and can even protect from it, are of great interest. This study investigated the protective effect of sodium tungstate against STZ-induced beta-cell damages by means of stereological methods. Sixty rats were divided into six groups: control (C), tungstate-treated control (TC), STZ-induced diabetic (D), STZ-induced diabetic rats were treated by sodium tungstate from 1 week before STZ injection (TDB), food-restricted diabetic (FRD), and diabetic rats treated with sodium tungstate 1 week after STZ administration (TDA). Stereological estimation of pancreas volume, islets volume density, volume-weighted mean islets volume and mass of beta cells, islets, and pancreas and total number of islets were done. Islets volume density, volume-weighted mean islets volume, and mass of beta cells, islets, and pancreas of TDB group was significantly higher than D, FRD and TDA groups ($P < 0.001$) and was comparable to controls (C and TC groups). Total number of islets, pancreas wet weight and volume did not show any significant changes between these groups ($P > 0.05$).

Results suggested that sodium tungstate preserves pancreatic beta cells from STZ-induced damages and diabetes induction in rats.

1. Introduction

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild et al., 2004).

In spite of the introduction of hypoglycemic drugs, diabetes and related complications continue to be a major medical problem. Therefore, new agents that are able to control and even to treatment diabetes are of great interest. In recent years, several inorganic elements have been described that mimic the effects of insulin or increase insulin action. These include derivatives of vanadium (Gao et al., 2006; Harati and Ani, 2004), chromium (Trumbo and Ellwood, 2006), molybdenum (Ozcelikay et al., 1996), cobalt (Vasudevan and McNeill, 2007) and zinc (Partida-Hernandez et al., 2006). Recent studies have also shown that sodium tungstate possess anti-diabetic activity in several diabetic animal models (Barbera et al., 1994; Barbera et al., 1997; Barbera et al., 2001). This element increases the effects of insulin both in vivo and in isolated cells and tissues (Li et al., 1995; Munoz et al., 2001; Fernandez-Alvarez et al., 2004). Report also indicated that tungstate regenerated pancreatic beta-cells population in neonatal STZ rats, a type 2 diabetes model (Kawasaki et al., 2004).

Type I diabetes mellitus results from selective destruction of the insulin producing beta cells in the pancreatic islets (Hassid and Abrahams, 1966). Protective effect of sodium tungstate on streptozotocin-induced beta-cells damages has not been investigated previously. In light of previous studies, that indicated sodium tungstate improves diabetes complication (Barbera et al., 1994; Barbera et al., 1997; Barbera et al., 2001; Li et al., 1995; Munoz et al., 2001; Fernandez-Alvarez et al., 2004), we have undertaken to evaluate pancreatic beta-cells protective effects of sodium tungstate in streptozotocin-induced type 1 diabetic rat by means of stereological methods.

Stereology is a technique that enables acquisition of data on number, volume, length, or surface area of identifiable objects in a three-dimensional structure by sampling in two dimensions. It provides a technique for quantifying objects on a slice from the structure, such as a histological specimen viewed under the microscope. It has the enormous virtue of having a rigorous mathematical foundation and rules for counting that give a reliable measure as well as an indication of precision. First-order stereology denotes estimating volume, surface area, length, or number of any biological object (Inuwa, 2005).

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doi:10.1016/j.micron.2008.02.014

2. Material and methods

2.1. Animals

The study was performed on mature normoglycemic male Wistar rats, weighing 200–220 g, which were separately housed in cages (one rat per cage). Animals were maintained in a room at 23 ± 2 °C, humidity 45–55% with a fixed 12-h artificial light period and allowed to eat and drink *ad libitium*. All animals were fed with standard rodent diet with the following composition (w/w): 20% protein, 3% fat, 2% fiber, 6% minerals and 69% starch and vitamins supplements. All animals received humane care, as outlined in the guide for the care and use of laboratory animals. Ethical committee of Zahedan University of Medical Sciences approved this study.

2.1.1. Induction of diabetes in animals

Type I diabetes was induced by a single intraperitoneal administration of streptozotocin (50 mg/Kg of body weight) in 0.15 M NaCl with 100 mM sodium citrate buffer (pH 4.5).

2.2. Experimental design

Sixty rats were divided into six following groups (n = 10):

(i) Control group (C): Rats of this group received standard rodent diet and tap water. After 1 week, they received IP vehicle (0.15 M NaCl with 100 mM sodium citrate buffer).
(ii) Tungstate-treated control group (TC): Rats of this group received tap water supplemented with 1–1.75 mg/ml sodium tungstate during the experiment.
(iii) Diabetic group (D): In this group diabetes was induced by IP injection of STZ.
(iv) Sodium tungstate-treated 1 week before STZ treatment (TDB): rats of this group received tap water supplemented with 1–1.75 mg/ml sodium tungstate at 1 week before STZ injection. Concentration of tungstate was gradually raised from 1 mg/ml to final concentration of 1.75 mg/ml by 5 weeks.
(v) Food-restricted diabetic group (FRD): Rats of this group received tap water and same amount of food as that consumed by TDB group. Because tungstate treatment was accompanied by a reduced weight gain, which may possibly influence glucose homeostasis (Kawasaki et al., 2004), a group of untreated diabetic rats (FRD) received a restricted amount of food to ensure a body weight gain similar to that of tungstate-treated animals (TDB). This amount was experimentally adjusted daily and was given them in two rations (one-third at 9.00 h and two thirds at 18.00 h).
(vi) Sodium tungstate-treated 1 week after induction of diabetes (TDA): rats of this group received tap water supplemented with 1–1.75 mg/ml sodium tungstate at 1 week after STZ injection and continued for 5 weeks.

The experiment was carried out for 5 weeks after STZ administration in diabetic groups.

2.3. Glucose and insulin measurement

At the end of the experiment, and after overnight fast, all animals were sacrificed under light ether anesthesia. Immediately blood samples were collected from tail vein. Blood glucose levels were measured by standard method of oxidase–peroxidase paired enzyme adapted for a RA 1000 analyzer (Technicon, USA), and serum insulin levels were determined by ultra sensitive rat insulin kit (DRG, France) using double-antibody enzyme-linked immunosorbent assay (ELISA).

2.4. Preparation of tissues

The pancreases were quickly removed, placed in cold saline solution and trimmed of adipose tissue, weighed, and immersed in modified Lillie's solution for 1 week at room temperature. After tissue processing and embedding in paraplast, each pancreas was exhaustively sectioned into 5 μm thick sections.

Physical randomization of tissue orientation was introduced by allowing pancreas samples to settle haphazardly in the embedding paraplast and the dispositions and orientations of islets are very variable thus the requirements of random location and isotropy for unbiased estimates are likely to be met (Mayhew, 1999).

Estimation of volume-weighted mean islet volume requires isotropic point sampled intercepts, which in turn demand either isotropic uniform random, or vertical plane sections. In this study, we used intercepts were generated on parallel Cavalieri sections with a fixed orientation, which in principle it may lead to biased results. As the islets are isotropic themselves, then the biasing impact will be low.

2.5. Staining method

Pancreatic sections were stained with modified aldehyde fuchsin histochemical method (Bancroft and Gamble, 2002).For this purpose sections rehydrated, oxidized in 1% potassium permanganate plus 0.5 ml concentrated sulfuric acid. Bleaching was done by 1% oxalic acid solution, and stained by aldehyde fuchsin for 1 h. Counter-staining was done by orange G and light green solution. Pancreas sections were mounted after dehydration in absolute ethanol and cleared in xylene. By this procedure, beta and alpha cells in islets of pancreas stain purple-violet and yellow, respectively.

2.6. Stereological study

2.6.1. Pancreas volume, islets volume density and total volume, total number of islets, volume-weighted mean islets volume

Ten to 12 sections of ~5 μm were sampled from each gland by systematic uniform random sampling (Howard and Reed, 1998). In order to project, the whole section image on the table, a BH2-Olympus light microscope with a projecting arm was used. Point counting using the Cavaliers principle was employed to estimate the volume of pancreas using the formula:

$$\mathrm{estV} = \frac{\sum_{i=1}^{m} P \cdot a/p \cdot \bar{t}}{M^2}$$

where estV is estimation of the volume of the pancreas, ΣP is the sum of the number of points landing within the pancreas profiles, a/p is the area associated with each point, t is the distance between sections and M is the magnification (Howard and Reed, 1998; Mandarim-de-Lacerda, 2003).

On each sampled section five to seven fields were selected in a systematic random manner by movement of the microscope's stage in X and Y directions with the aid of vernier scale of the stage of a projection microscope (Olympus, Japan). A transparent test system was then superimposed on these fields and points hitting the various components of the gland were counted at a final magnification of 32. Then an estimate of the volume density, V_v, of the components in the reference space was obtained using: $\mathrm{estV_v} = P(\mathrm{part})/P(\mathrm{ref})$, where P(part) and P(ref) are the number of test points falling in all structure profiles and in the reference space, respectively (Howard and Reed, 1998; Gundersen and Jensen, 1985; Gundersen et al., 1988).

In order to estimate the absolute volume of a part, the volume density of that part is multiplied by the reference volume (Howard and Reed, 1998).