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Pancreatic Islet Beta Cell Protective Effect of Oral Vanadyl Sulphate in Streptozotocin-induced Diabetic Rats, an Ultrastructure Study

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Abstract: We sought to determine the ultrastructure of pancreatic islet beta cells of streptozotocin-induced diabetic rats treated with oral vanadyl sulphate. Diabetes was induced in male Wistar rats by intravenous injection of 40 mg kg^{-1} streptozotocin. The same volume of normal saline was injected in sham animals. Animals were divided into treated and control groups. Vanadyl sulphate was added to the drinking water of the treated animals at a concentration of 1 mg mL^{-1} up to three months. Two months after vanadyl sulphate withdrawal animals were killed. Ultrastructure of islet beta cells were studied by transmission electron microscope. In diabetic treated rats plasma glucose and fluid intake returned to normal levels within three months while control animals remained diabetic. Well granulated cytoplasm, well developed endoplasmic reticulum , increase in the number of immature granules in the cytoplasm with no clear signs of cell injury were found in the islet beta cells of diabetic treated rats. Lymphocyte filteration, nuclear picnosis, cytoplasmic vacuolization were found frequently in the islet beta cells of untreated diabetic rats. In conclusion as was evident in thin sections of panceatic islet beta cells of treated diabetic rats in this study, vanadyl sulphate through preserving islet beta cells structure and ultrastructure contributes in reversing diabetic signs and symptoms in streptozotocin induced diabetic rats.

Key words: Streptozotocin, vanadyl sulfate, plasma glucose, beta cell, ultrastructure, diabetes

INTRODUCTION

The pathogenesis of the type 1 diabetes (IDDM) is characterised by immune-mediated destruction of insulin-producing pancreatic beta cells (Tsai *et al.*, 2008; Yoon and Jun, 1999) culminating in a state of hyperglycemia. Chronic hyperglycemia is associated with alterations in β-cell mass and function and risk of microvascular disease and nephropathic complications (Poitout and Robertson, 2008). There is some evidence that a maladaptive response to the hyperglycemia contributes to the chronic complications of diabetes (Chan *et al.*, 2006). The disease can be prevented by preventing complete autoimmune destruction of islet β cells and preservation/restoration of B-cell mass and function (Cernea and Pozzilli, 2008; Bach and Chatenoud, 2001).

Streptozotocin, an analog of N-acetyl glucosamine, has been used in many studies for induction experimental model of type 1 diabetes (Cam *et al.*, 1997; Ashok *et al.*, 2010). Mechanism of action of Streptozotocin in inducing diabetes is not completely understood. Inhibition of pancreatic islet beta cell *O*-GlcNAc-selective *N*-acetyl-b-d-glucosaminidase, which removes O-GlcNAc from intracellular proteins, has been proposed as the possible mechanism of STZ diabetogenic toxicity (Konrad *et al.*, 2001).

Vanadium, a transitional element, with the symbol V and atomic number 23 which can be found in various oxidative states, can mimic most of the biological effects of insulin, including improving glucose homeostasis, normalizing plasma lipid profile and enhancing insulin sensitivity in experimental models of diabetes mellitus (Crans, 2000; Badmaev *et al.*, 1999; Thompson and Orvig, 2006). Vanadium can prevent the exhaustion of pancreatic insulin stores in genetically obese hyperglycemic insulin-resistant ob/ob mice (Brichard *et al.*, 1990). Trophic effect has been reported in the pancreatic islets of Vanadyl Sulphate (VS) treated streptozotocin (STZ) diabetic rats (Dehghani *et al.*, 1999). To our knowledge little research has done about the ultrastructure of islet beta cells of diabetic animals treated

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with vanadium compounds. The present study was designed to see the effect of the oral VS treatment on the amelioration of diabetic signs and in order to investigate the ultrastructure of pancreatic islet beta cell of STZ diabetic rats after VS treatment and withdrawal using transmission Electron Microscopy (EM).

MATERIALS AND METHODS

Materials: Vanadyl sulphate ($VOSO_4$, H_2O) was from Aldrich Chemical Co. USA. Streptozotocin was purchased from Pharmacia and Upjohn Company (Kalamazoo, USA). Collagenase type B was from Boehringer Mannheim Corp., Indianapolis. Hanks' Balanced Salt Solution (HBSS), liquid (pH = 7.4) was prepared from its components in the laboratory. Philips transmission electron microscope from Bagiatollah Hospital, Tehran, Iran was used for electron microscopic studies. Plasma Glucose was measured by glucose kit (Ziest Chimi Diagnostics, Tehran, Iran) using Spectrophotometer UV-3100/3100 sec, Shimadzu, Japan.

Treatment and maintenance of rats: Male Wistar rats weighing between 180 to 220 g, were purchased from Razi institution (Karaj, Iran) in 2002. Rats were maintained under standard condition and had free access to food. Diabetes was induced by a single intravenous injection of freshly prepared solution of streptozotocin (40 mg kg^{-1} b.wt., i.v.) in 0.1 M citrate buffer (pH 4.5) through lateral tail vein ([D], n = 64). One group of normal rats was injected with the same amount of normal saline through lateral tail vein as STZ solution injected in diabetic rats and was considered as sham group ([N], n = 28). Blood samples for analyses were taken from the nicked tail vein in the micro centrifuge tubes. Plasma separated by centrifugation and plasma glucose measured immediately after plasma separation by glucose kit using spectrophotometer. Animals were considered diabetes when plasma glucose measured one week after STZ injection was greater than 400 mg dL^{-1}.

Diabetic and sham rats were divided into Control © and Treated (T) groups. VS was provided in the drinking water to the Diabetic Treated (DT) and Normal Treated (NT) groups began seven days after STZ injection in the concentrations of 0.5 mg mL^{-1} for one week and 1 mg mL^{-1} for the rest of the study up to three months. Control groups received top water during the same period. All group of animals received top water for the next two months. Plasma glucose and fluid intake monitored frequently during and after withdrawal of the treatment. All animals were killed with an overdose of ether at the end of study.

Ultrastructural study: Islets were prepared by collagenase digestion based on method used by Lacy and Kostianovsky (1967). For this purpose first the common bile duct was cannulated and pancreata were distended with 10 mL HBSS. The pancreas was carefully dissected from surrounding tissue and placed in cooled HBSS. Pancreas was cut into fragments and digested immediately in HBSS solution with 2 mg mL^{-1} type B Collagenase. We added fresh PBS to digested islet solution to 15 mL and centrifuged for 5 min at 1000 rpm. Supernatant was discarded and pellet was washed twice with HBSS and resuspended in 15 mL HBSS. Islet containing Hanks solution was transferred to a 15 mL dark stained Petri dish and islets were handpicked under dissecting microscope. Picked islets were fixed in 0.1 M phosphate-buffered (pH 7.4) 2.5% glutaraldehyde for 2 h at room temperature, post fixed in 1% OsO_4 for 1 h at 4°C, dehydrated in ethanol and embedded in Epon. Areas of interest for electron microscopic analysis were chosen from semi thin sections. Ultra thin sections were stained with uranyl acetate and lead citrate and observed with a Philips transmission electron microscope.

Statistical analysis: Data was analyzed using SPSS software and presented as the Means±SE. For testing the homogeneity of variances Bartlett's test was used. In case of multiple comparisons statistical significance of differences was calculated with One-way Analysis of Variance (ANOVA) followed by Tukey as a post hoc test.

RESULTS

Glycaemia and fluid intake: Plasma glucose and fluid intake in different groups of animal during the period of study are shown in Table 1 and 2, respectively. In the

Table 1: Plasma glucose (mg dL^{-1}) in different groups of animals during the period of study

Groups	Day		
	7	90	120
Diabetic control (n)	526±21[a] (64)	478±23[b] (22)	456±27[b] (14)
Diabetic treated (n)		124±12 (64)	129±12 (14)
Normal control (n)	110±8 (28)	118±10 (14)	122±11 (14)
Normal treated (n)		110±8 (28)	112±9 (12)

Data are Mean±SE. a: p<0.01 vs. normal group at the same day b: p<0.01 vs. respective treated group at the same day

Table 2: Fluid intake (mL day^{-1}) in different groups of animals during the period of study

Groups	Day		
	7	90	120
Diabetic control (n)	142±12[a] (64)	169±13[b] (22)	151±13[b] (14)
Diabetic treated (n)		25±4 (64)	26±5 (14)
Normal control (n)	24±4 (28)	27±3 (14)	25±3 (14)
Normal treated (n)		21±4 (28)	20±4 (12)

Data are Mean±SE. a: p<0.01 vs. normal group at the same day b: p<0.01 vs. respective treated group at the same day
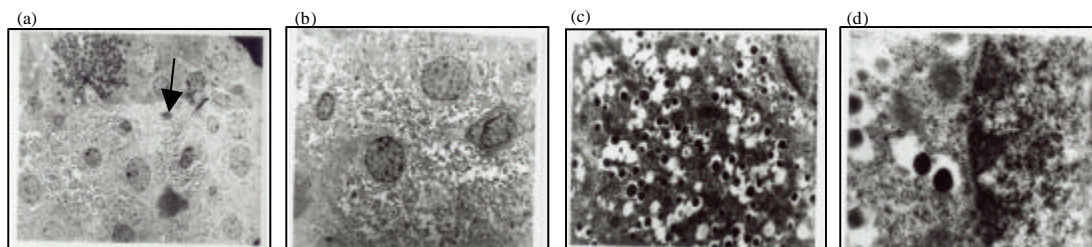
Fig. 1: Thin sections of pancreatic islet and islet beta cells of normal control rats. Left (a) arrow shows border of islet and exocrine cells. (d) Islet beta cells with clear beta cell granules and normal chromatin distribution (X = 38000)
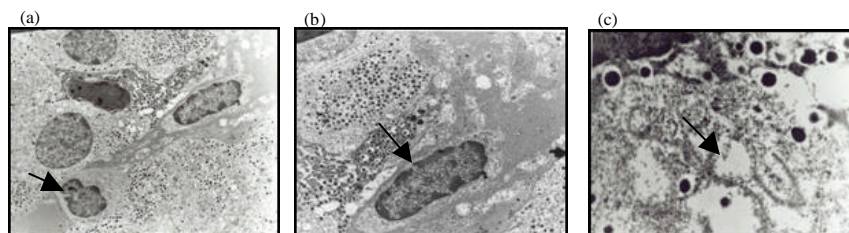

Fig. 2: Thin sections of pancreatic islets of untreated diabetic (DC) rats. Lymocyte infilteration (a), chromatin condensation and marginalization toward priphery in nucleus (a) and vacoulation of cytoplasm are shown by arrows [(c) x = 29000)]
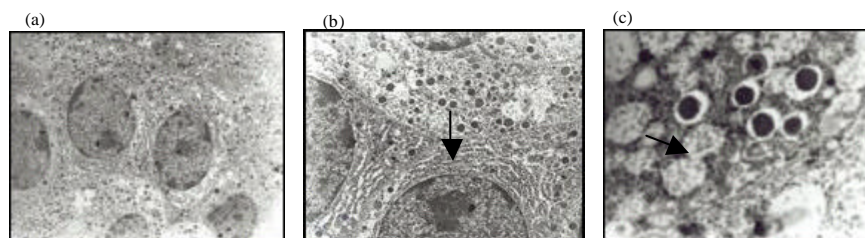

Fig. 3: Thin sections of pancreatic islet beta cells of VS treated diabetic rats. Well developed endoplasmic reticulum [b (X = 10500)] and presence of immature granules in the cytoplasm are shown by arrows [c (x = 38000)]

majority of diabetic treated rats plasma glucose and fluid intake that increased approximately five folds one week after STZ injection dropped to normal levels by VS treatment within three months and remained normal, two months after VS withdrawal, until they were killed. A small number of VS treated diabetic animals remained hyperglycemic and were omitted from the study. Even in those animals who responded very well to treatment and their plasma glucose and fluid intake was normal in the presence of VS, we observed returning diabetic symptoms if we withdrew VS from the drinking solution in the first few weeks of treatment. Untreated diabetic rats remained both hyperglycemic and polydipsic during the period of study and some of those animals showed mono lateral and bilateral cataract. We lost some of the DC and DT animals during the period of study. A small decrease in plasma glucose and fluid

intake was observed in NT animals in comparison to NC animals but it was not significant statistically.

Electron microscopy: Islet beta cells were identified in thin sections morphologically according to the criteria used by Sacchi TB (Sacchi and Bani, 1985) including the position of islet beta cells and characteristics of granules, dense core and electron lucent halo spaces. Figure 1-3 show the ultrastructure of islets of normal control, diabetic control and diabetic treated animals respectively. In the islets of VS treated diabetic rats (Fig. 3), the size of beta cells was smaller than normal but the number of beta cells and the granules within cytoplasm, especially immature granules was increased when compared with normal islet beta cells. Well developed endoplasmic reticulum was found in the cytoplasm of beta cells in DT animals when compared with control animals. Nucleus and

chromatin in DT animals did not show any obvious changes as compared to control animals. Cytoplasm as well as nuclei of islet beta cells of diabetic control animals showed typical signs of degeneration, border of cells was not clear and nuclear chromatin was irregularly condensed (Fig. 2). Reduced secretory granules, margination of granules toward cell periphery, nuclear pyknosis and cytoplasmic vacuolization was found in the cytoplasm of beta cells of DC animals. Lymphocyte infiltration was found frequently among islet cells in DC animals. No obvious changes were found between normal treated and control animal islet beta cells.

DISCUSSION

The result of this study showed that VS treatment reversed diabetic states in the majority of the rats made diabetic by moderate dose (40 mg kg^{-1}, i.v.) of STZ. VS treated diabetic rats remained normoglycemic two months after VS withdrawal, until they were killed. Well preservation of pancreatic islet beta cells ultrastructure including normal chromatin distribution, well developed endoplasmic reticulum and well granulation of cytoplasm which was found in electron microscopic studies of islet beta cells indicated that amelioration of diabetic signs was along with preservation of islet ultrastructure.

Our previous study showed that VS treatment could not reverse diabetic state in severe diabetes induced by 50-55 mg kg^{-1} (i.v.) STZ in rats but reduced the amount of intraperitoneal insulin needed to induce normoglycmia (Dehghani *et al.*, 1997). The result of current study showed that VS treatment can reverse signs and symptoms of moderate diabetes induced by STZ in rats without any signs of recurrence long after vanadium withdrawal from the drinking solution of diabetic rats. With respect to the distribution of vanadium in plasma and other tissues and half life for elimination of vanadium from the bodies of vanadium-fed rats (Ramanadham *et al.*, 1991; Setyawati *et al.*, 1998; Yasui *et al.*, 2000), vanadium concentration in animal tissues two months after VS withdrawal should be around pre treatment levels while treated diabetic animals still did not show any obvious signs of diabetes recurrence.

The exact mechanism of vanadium in reversing diabetic state in experimental diabetic animals is not clear. Even though vanadium compounds affect the activity of many enzymes involved in metabolic pathways and mimic the effect of insulin, those effects are not sufficient for long-term reversal of the diabetic state. Compensatory β cell regeneration can occur in response to STZ injection but it cannot reverse hyperglycemia in severe diabetes induced by higher doses of STZ (Rankin and Kushner, 2009; Plachot *et al.*, 2001). Pancreatic tissues in young rodents have the capacity of remodeling and STZ injection in newborn rats will be followed by remission from neonatal diabetes due to β cell regeneration (Cam *et al.*, 1997; Wang *et al.*, 1994; Portha *et al.*, 1989). Adult β cells have a limited proliferative capacity but even in adult rats β cell neogensis can be induced through duct ligation or gene expression (Bonner-Weir *et al.*, 1993; Bertelli and Bendayan, 1997; Rosenberg *et al.*, 1996a, b; Yamamoto *et al.*, 2000; Vinik *et al.*, 1992; Sharma *et al.*, 1999). There is some evidence for presence of islet stem cells in the pancreatic ducts and islets (Lechner and Habener, 2003; Gmyr *et al.*, 2000).

Possible mechanisms of vanadium action in restoring islet beta cells and reversing diabetic symptoms in STZ diabetic rats are reducing hyperglycemia and its deleterious effect on β cells, potentiating regeneration response to the damaging effects of STZ and inducing islet neogensis from islet stem cells in the pancreas by mechanisms like inhibitory effect on phosphatase or by activating transcription factors in the potential islet precursors cells like ductal epithelial cells and acinar cells. Increase in the number of immature granules and well developed rough endoplasmic reticulum in the islet beta cells of VS treated diabetic rats as found in this study led us to propose a role for hyper function of the remaining beta cells under the influence of VS treatment as another possible mechanism of VS action in reversing diabetic signs.

Part of the result of this study apparently looks to be not in agreement with our previous reported results that VS treatment did not reverse the diabetic states in diabetes made by intravenous injection of 50 mg kg^{-1} STZ (Dehghani *et al.*, 1997). Even though pancreatic β-cells are more sensitive to STZ than any other cells, STZ as a diabetic agent is not specific and can damage other cells including exocrine acinar cells (Azza Attia, 2009) which probably is the place of some islet stem cells (Gmyr *et al.*, 2000). In our previous study we used higher doses of STZ and the severity of diabetes and following hyperglycemia probably caused loosing both islet beta cells and its potential precursors in the pancreas including acinar cells and led to permanent failure of rescue or induction effects of vanadium in those diabetic rats. In that case vanadium cannot do anything with plasticity of endocrine pancreas and only insulin mimetic effects of vanadium can make animal's tissue sensitive to endogenous or exogenous insulin.

Based on the result of this and our previous studies in this regard, vanadium compounds can reverse diabetic

symptoms through potentiating the effect of insulin and preserving islet beta cells and have beneficial effect in both moderate and severe diabetes induced by STZ in rats. However their outcomes are affected by the severity of diabetes, duration of treatment, vanadium dose and physiological differences between animals. Insulin mimetic effects of vanadium make it a suitable element for ameliorating of diabetic symptoms in type II diabetes patients which have enough endogenous insulin and in decreasing the amount of insulin needed to correct hyperglycemia in chronic type 1 diabetes patients. Protective or regenerative effects of vanadium give the capability to this trace element to be used in those who can be screened for type 1 diabetes and before the complete exhaustion of pancreatic islet beta cells or in newly diagnosed type 1 diabetic patients.

As a conclusion, VS treatment can lead to long term reversal of diabetic state in moderate diabetes induced by STZ in adult rats. Preventing the full destruction of injured β cells, preserving a portion of islet β cells that survived STZ toxicity, hyper function of the remaining healthy beta cells, preventing T cell homing into the islets and β cell regeneration from beta cell precursors in the endocrine or exocrine portion of pancreas are possible mechanisms beyond long term reversal of diabetic state under the influence of vanadium compounds. Luck of enough reliable information in human trials about side effects of prolonged administration of vanadium indicates limitation of its application for treating humans with diabetes mellitus. More investigations are needed to better define the mechanisms behind protection of islet beta cells from the destructive effects of moderate dose of STZ or (possible) regeneration or neogensis of new islets beta cells by vanadium compounds.

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