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Vanadyl sulfate administration protects the streptozotocin-induced oxidative damage to brain tissue in rats

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Abstract

Diabetes mellitus manifests itself in a wide variety of complications and the symptoms of the disease are multifactorial. The present study was carried out to investigate the effects of vanadyl sulfate on biochemical parameters, enzyme activities and brain lipid peroxidation, glutathione and nonenzymatic glycosylation of normal- and streptozotocin-diabetic rats. Streptozotocin (STZ) was administered as a single dose (65 mg/kg) to induce diabetes. A dose of 100 mg/kg vanadyl sulfate was orally administered daily to STZ-diabetic and normal rats, separately until the end of the experiment, at day 60. In STZ-diabetic group, blood glucose, serum sialic and uric acid levels, serum catalase (CAT) and lactate dehydrogenase (LDH) activities, brain lipid peroxidation (LPO) and nonenzymatic glycosylation (NEG) increased, while brain glutathione (GSH) level and body weight decreased. In the diabetic group given vanadyl sulfate, blood glucose, serum sialic and uric acid levels, serum CAT and LDH activities and brain LPO and NEG levels decreased, but brain GSH and body weight increased.The present study showed that vanadyl sulfate exerted antioxidant effects and consequently may prevent brain damage caused by streptozotocin-induced diabetes. (Mol Cell Biochem **286:** 153–159, 2006)

Key words: brain, diabetes mellitus, rat, serum, vanadyl sulfate

Introduction

Diabetes mellitus (DM), characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, nerves, livers and blood vessels, is the most common endocrine disorder [1]. Although the underlying mechanisms of diabetic complications remains unclear, much attention has been focused on the role of oxidative stress.

It has been suggested that increased oxidative stress is a widely accepted participant in the development of diabetic complications [2, 3]. It is clearly described that diabetes mellitus is always associated with an increased production of free radicals [4, 5]. Therefore, the antioxidant defenses are impaired in diabetes mellitus [6]. Vanadium is an essential trace element belived to be important for normal cell function and development in mammals [7]. Vanadium and vanadium compounds are responsible for insulin-like activity and can mimic the action of insulin through alternative signaling pathways [8].

The aim of this study was to investigate the biochemical effects of administration of vanadyl sulfate on the brain of normal and STZ- diabetic rats.

Materials and methods

Experimental animals

The experiments were reviewed and approved by the Animal Care and Use Institute's Committee of Istanbul University.

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In this study, male, 6-6.5 months old Swiss albino rats were used. Rats were fed with pellet chow and tap water *ad libitum*. All rats were clinically healthy. The animals were randomly divided into four groups: Group I: intact animals (control) ($n = 13$); Group II: animals given vanadyl sulfate (control) ($n = 5$); Group III : streptozotocin (STZ)-diabetic animals ($n = 11$); and Group IV: STZ-diabetic animals given vanadyl sulfate ($n = 11$) .Vanadyl sulfate was given by gavage technique to rats in a dose of 100 mg/kg every day for 60 days.

Induction of diabetes

Experimental diabetes was induced by intraperitoneal injection of STZ in a single dose of 65 mg/kg body weight [9]. STZ was dissolved in a freshly prepared 0.01 M citrate buffer (pH 4.5).

Biochemical assays

In this study, biochemical investigations were made in blood, serum and brain tissue. Blood samples from rats were collected from the tail vein at 60th day. In all samples, 18-h-fasting blood glucose levels were determined by o-toluidine method [10]. At day 60, cardiac blood samples were taken from all rats under ether anesthesia. Serum sialic acid was estimated by Warren's method with slight modifications [11]. Uric acid was estimated by Caraway's method [12]. Catalase activity (CAT) was measured according to Aebi [13]. Lactate dehydrogenase (LDH) activity was assayed according to the method used by Wroblewski [14].

At the end of the experimental period, brain tissues were taken from animals under ether anesthesia after an overnight fast. Brain tissues were homogenized to10% (w/v) homogenate with cold 0.9% saline using a glass equipment. Tissue homogenates were centrifuged and the clear supernatants were used for glutathione (GSH), nonenzymatic glycosylation (NEG), lipid peroxidation (LPO), and protein analysis. Brain glutathione (GSH) levels were determined with Beutler's method using Ellman reagent [15]. LPO levels in brain homogenates were estimated by the method of Ledwozyw [16]. NEG levels were determined by thiobarbituric acid method [17].Protein contents in the supernatants were determined by Lowry's method using bovine serum albumin as standard [18].

Statistical analysis

The results were evaluated using an unpaired t-test and ANOVA variance analysis the NCSS statistical computer package.

Results

Changes in body weight and blood glucose levels in the control and experimental groups are shown in Table 1. At the end of the study, body weight in diabetic group was significantly lower when compared with the other groups ($P_{ANOVA} = 0.0001$). Treatment with vanadyl sulfate improved the weight gain as compared to diabetic rats (Table 1). A significant difference in the blood glucose levels of all groups was observed at the end of experiment ($P_{ANOVA} = 0.0001$). The blood glucose levels were significantly increased in STZ diabetic rats as compared to control rats ($^{b}P_{t\text{-test}} = 0.0001$). Oral administration of vanadyl sulfate significantly decreased the blood glucose levels in diabetic rats ($P_{t\text{-test}} = 0.001$).

A considerable difference in the sialic acid levels of all groups was observed ($P_{ANOVA} = 0.0001$), (Table 2). Serum sialic acid levels were significantly higher in diabetic groups when compared to controls ($^{a}P_{t\text{-test}} = 0.0001$). Oral

Table 1. Mean levels of body weight and blood glucose parameters for all groups

Groups	n	Weight (g)*	$P_{t\text{-test}}$	Blood glucose (mg /dl)*	$P_{t\text{-test}}$
Control	13	283.48 ± 30.35		69.05 ± 9.15	
			0.049		0.004
Control+Vanadyl Sulfate	5	251.91 ± 20.41		86.40 ± 11.03	
Diabetic	11	171.70 ± 34.67[a]		323.39 ± 131.29[b]	
			0.084		0.001
Diabetic+Vanadyl Sulfate	11	199.16 ± 36.20		131.68 ± 72.30	
P_{ANOVA}		0.0001		0.0001	

*Mean ± SD.
n = number of animals.
[a,b] $P_{t\text{-test}} = 0.0001$ versus to control group.

Table 2. Mean levels of serum sialic acid and uric acid parameters for all groups

Groups	n	Sialic Acid (mmol/l)*	$P_{t\text{-test}}$	Uric acid (mg/dl)*	$P_{t\text{-test}}$
Control	13	2.54 ± 0.07	0.0001	1.33 ± 0.29	0.876
Control+Vanadyl Sulfate	5	2.79 ± 0.09		1.35 ± 0.15	
Diabetic	11	3.08 ± 0.05[a]	0.0001	1.97 ± 0.09[b]	0.0001
Diabetic+Vanadyl Sulfate	11	2.81 ± 0.05		1.37 ± 0.09	
P_{ANOVA}		0.0001		0.0001	

*Mean ± SD.
n = number of animals.
[a,b] $P_{t\text{-test}} = 0.0001$ versus to control group.

Table 3. Mean levels of serum catalase and lactate dehydrogenase activities for all groups

Groups	n	Catalase (U/ml)*	$P_{t\text{-test}}$	Lactate Dehydrogenase (U/ml)*	$P_{t\text{-test}}$
Control	13	2020.36 ± 209.59	0.002	4933.00 ± 115.47	0.0001
Control+Vanadyl Sulfate	5	2704.13 ± 67.47		7650.00 ± 212.13	
Diabetic	11	2818.35 ± 289.66[a]	0.066	7000.00 ± 50.00[b]	0.0001
Diabetic+Vanadyl Sulfate	11	2534.17 ± 72.74		5925.00 ± 150.00	
P_{ANOVA}		0.0001		0.0001	

*Mean ± SD.
n = number of animals.
[a,b] $P_{t\text{-test}} = 0.0001$ versus to control group.

administration of vanadyl sulfate decreased considerably the serum sialic acid levels in diabetic rats ($P_{t\text{-test}} = 0.0001$), (Table 2). A significant difference in the serum uric acid levels of all groups was also observed ($P_{ANOVA} = 0.0001$), (Table 2). Serum uric acid levels were remarkably higher in diabetic groups when compared with controls ($^{b}P_{t\text{-test}} = 0.0001$). Oral administration of vanadyl sulfate decreased notably the serum uric acid levels in diabetic rats ($P_{t\text{-test}} = 0.0001$).

A considerable increase was observed in the serum catalase activity ($^{a}P_{t\text{-test}} = 0.0001$). Administration of vanadyl sulfate caused a decrease in serum catalase activity in the STZ-diabetic rats. Serum LDH activities were remarkably increased in the diabetic group compared with control groups ($^{b}P_{t\text{-test}} = 0.0001$). Administration of vanadyl sulfate caused a notable reduction in serum LDH activity in the diabetic group ($P_{t\text{-test}} = 0.0001$), (Table 3).

Table 4 shows the contents of LPO, GSH and NEG in the brain tissues of control and diabetic animals. A significant difference in the brain LPO levels of all groups was observed ($P_{ANOVA} = 0.0001$). Brain LPO levels were significantly higher in diabetic groups when compared with controls ($^{a}P_{t\text{-test}} = 0.008$). Vanadyl sulfate given to the diabetic rats lowered the brain LPO levels in a considerable manner when compared with diabetic rats ($P_{t\text{-test}} = 0.0001$). Brain GSH levels were obviously reduced in the diabetic animals as compared with other groups ($P_{ANOVA} = 0.001$). In diabetic animals treated with vanadyl sulfate, the brain GSH levels markedly increased when compared with the diabetic group ($P_{t\text{-test}} = 0.005$). In the diabetic rats, NEG levels were significantly higher than those of the other groups ($P_{ANOVA} = 0.0001$). Administration of vanadyl sulfate was found to reduce brain NEG levels in diabetic rats ($P_{t\text{-test}} = 0.0001$).

Discussion

Diabetes mellitus is a major health problem in developed and developing countries [19]. The major types of diabetes are characterized by hyperglycemia, abnormal lipid and protein metabolisms, along with spesific long-term complications that irreversibl affect the eyes, liver, kidney, and nervous system [20, 21].

STZ-induced diabetes is characterized by severe loss in body weight due to increased catabolic reactions leading to muscle waste [22–24]. The loss in body weight also seen in the present study, was partly prevented by vanadyl sulfate (Table 1) and thus could be attributed to the antidiabetic role of the compound.

gen used in brain converted CO_2 and water, some little amount of oxygen forms free radicals like O_2^{-}, H_2O_2 and OH^{-}. The existance of polyunsaturated fatty acids which are targets of those of the free radicals make this organ more sensitive to oxidative damage [44, 45]. There are various antioxidant mechanisms in brain neutralizing the harmful effects of free radicals, yet in diabetes, loss of efficiency of antioxidants mechanism and alterations in the electron transfer chain in mitochondria is reported [44].

Hyperglycemia develops after streptozotocin administration and stimulates the production of advanced glycosylated end-products, enhances the polyol pathway and activates protein kinase C [2]. These conditions might lead to increased generation of ROS, such as superoxide anion (O_2^{-}) [46], which rapidly reacts with NO leading to the formation of ONOO, which is highly oxidant and capable of damaging several biological molecules [47]. The peroxidation of polyunsaturated fatty acids with the concomitant formation of aldehydic products is an indicator that OH^{-} has been formed [48]. In the present study, streptozotocin administration resulted in a significant increase in TBARS (Table 4), indicating an increased oxidative stress due to overproduction of ROS in brain [49]. The significant decrease of lipid peroxides in brain of vanadyl sulfate-treated diabetic rats and increased activities of enzymic and non-enzymic antioxidants in brain suggest that vanadyl sulfate reduce oxidative stress by quenching free radicals. It may be concluded that in diabetes, brain tissue was more vulnerable to oxidative stress and showed increased lipid peroxidation. The increased oxidative stress in diabetes [2, 50] produces oxidative damage in many regions of rat brain including the hippocampus. Oxidative damage in rat brain is increased by experimentally induced hyperglycemia [51]. Oxidative damage to various brain regions constitute into the long term complications, morphological abnormalities and memory impairments [52, 53]. The above observation shows that the vanadyl sulfate possess antioxidant activity, which could exert a beneficial action against pathological alterations caused by the presence of free radicals in STZ-diabetes.

One of the most important intracellular antioxidant system is the glutathione redox cycle. Glutathione is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in the cell metabolism [54]. GSH acts as an antioxidant *in vivo* and its decrease was reported in diabetes mellitus [55]. In this study, we have observed significant decrease in GSH levels in brain during diabetes (Table 4). The decrease in GSH levels represents increased utilization due to oxidative stress [56]. Administration of vanadyl sulfate increased the content of GSH in the brain of diabetic rats, thus protecting cellular proteins against oxidation through glutathione redox cycle and also directly detoxifying ROS generated from exposure to STZ [57]. The increase in GSH content in diabetic rats treated with vanadyl sulfate indicates an adaptive mechanism in response to oxidative stress.

Nonenzymatic protein glycosylation, resulting from direct reaction between reducing sugars and primary amino groups, is increased during diabetes and is believed to contribute to the long term complications of the disease [58]. NEG of the brain proteins causes alteration in their structures and functions. The levels of NEG were found to be increased in the STZ- diabetic groups with respect to controls (Table 4). Various means of preventing this increase have been investigated both *in vitro* and *in vivo* [8]. It has been reported that GSH, glycine, and various antioxidant vitamins, and trace elements such as vitamins C, and E, lipoic acid, vanadium, selenium, zinc, and chromium prevent the increase of tissue NEG levels [59]. In our study, we found a significant increase in NEG of the brain protein in diabetic rats. The administration of vanadyl sulfate reduced NEG levels in diabetic rats (Table 4).

Vanadium is known to be a catalyst that has been reported to induce ROS generation *in vitro*, as well as its features of lipid peroxidation and oxidative damage in experimental models [60]. Vanadium compounds may behave as antioxidants and pro-oxidants, depending on dose and experimental conditions [61]. In our results, STZ-induced diabetes caused a significant decrease in brain GSH and increase in NEG of the brain protein and brain LPO. The administration of vanadyl sulfate to STZ diabetic rats reduced NEG and LPO turned GSH toward its normal values. These results suggest that the administration of vanadyl sulfate may have a protective effect against brain tissue damage in pro-oxidant conditions during diabetes.

As a result, it might be concluded that vanadyl sulfate showed an improvement in all biochemical parameters and it could have a protective damage caused by diabetes on brain tissue.

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