Ameliorative effect of vanadium on oxidative stress in stomach tissue of diabetic rats

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Abstract

Between their broad spectrum of action, vanadium compounds are shown to have insulin mimetic/enhancing effects. Increasing evidence in experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes and on the onset of diabetic complications. Thus, preventive therapy can alleviate the possible side effects of the disease. The aim of the present study was to investigate the effect of vanadyl sulfate supplementation on the antioxidant system in the stomach tissue of diabetic rats. Male Swiss albino rats were randomly divided into 4 groups: control; control+vanadyl sulfate; diabetic; diabetic+vanadyl sulfate. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ; 65 mg/kg body weight). Vanadyl sulfate (100 mg/kg body weight) was given daily by gavage for 60 days. At the last day of the experiment, stomach tissues were taken and homogenized to make a 10% (w/v) homogenate. Catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), myeloperoxidase (MPO), carbonic anhydrase (CA), glucose-6-phosphate dehydrogenase (G6PD) and lactate dehydrogenase (LDH) activities were determined in the stomach tissue. CAT, SOD, GR, GPx, GST, CA, G6PD and LDH activities were increased in diabetic rats when compared to normal rats. Vanadium treatment significantly reduced the elevated activities of GR, GPx, GST compared with the diabetic group whereas the decreases in CAT, SOD, CA, G6PD and LDH activities were insignificant. No significant change was seen for MPO activity between the groups. It was concluded that vanadium could be used for its ameliorative effect against oxidative stress in diabetes.

KEY WORDS: vanadium, diabetes, stomach, oxidative stress, antioxidant

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficency of secretion or action of endogenous insulin. While exogenous insulin and other medications can control many aspects of diabetes, numerous complications affecting several tissues are common and are extremely costly in terms of longevity and quality of life [1]. Increasing evidence in experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles, increased lipid peroxidation and development of insulin resistance. The consequences of oxidative stress can promote the development of complications of diabetes mellitus [1]. The oxidative effect of diabetes on stomach tissue is demonstrated by the impairment of some biochemical parameters [2-4]. Vanadium derivatives have been shown to possess insulin mimetic and antidiabetic activities in animal models of type 1 and type 2 diabetes mellitus as well as in a small number of diabetic human subjects [5-8]. However, despite numerous studies during the past decade, the mechanism(s) by which vanadium mediates its *in vivo* antidiabetic effects are not well understood [9]. In a review, on considering the effects of vanadium on carbohydrate and lipid metabolisms, Cam et al. [10] concluded that vanadium acts selectively and by enhancing rather than by mimicking the effects of insulin *in vivo*. There are also different views on the efficacy of vanadium in the control of hyperglycaemia. Smith et al. [11] states that there is no rigorous evidence that oral vanadium supplementation improves glycaemic control in type 2 diabetes and that the routine use of vanadium for this purpose cannot be recomended. On the other

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hand, numerous studies report the glucose lowering effect of vanadium salts and propose vanadium complexes as potential agents in the aid of glycaemic control [12-15]. As a result of its more common occurrence in the environment, vanadium is absorbed by plants and travels along the food chain into the body of animals and humans. Although there is no strong evidence that vanadium is an essential trace element for human, a necessary dose of 10 μg/daily in humans is reported [16]. There are contradictory reports on whether vanadium compounds have toxic effects or not. Domingo et al. [17] reported severe toxic side effects of vanadium on streptozotocin-induced diabetic rats, while Dai et al. [18] concluded that vanadyl sulphate at antidiabetic doses is not significantly toxic to rats following a one-year administration. It is known that vanadium is poorly absorbed in the gastrointestinal tract and rapidly excreted by kidneys [19] which reduces its toxic effects and accumulation in tissues, but also limits its therapeutic efficacy. The accumulation of vanadium in tissues follows the order; bone > kidney > liver > spleen > intestines > stomach > muscle > testis > lung > brain [20]. For several years inorganic vanadium compounds, such as sodium orthovanadate and vanadyl sulfate were used in both animal and human studies. Although these compounds were shown to be glucose-lowering agents, their side effects, mainly gastrointestinal discomfort [13], limited their use as therapeutic agents [9]. In this study, the ameliorative potential of oral administration of vanadium on the stomach tissue of streptozotocin (STZ)-diabetic rats via its effect on antioxidant system enzymes was investigated in order to elucidate the mechanism by which this trace element exerts its beneficial effects.

MATERIALS AND METHODS

Animals and treatment

The experiments were reviewed and approved by the Animal Care and Use Institute Committee of Istanbul University. In this study, 6-6.5 months old male Swiss albino rats were used. Animals were acclimatized to their environment for one week prior to experimentation. The animals were housed in a room with a 12 h light/dark cycle at about 22°C and fed on standard diet with *ad libitum* access to drinking water. The rats were randomly divided into 4 groups: Control: non-diabetic intact animals (n=13), Control+Va: control animals given vanadyl sulfate (n=5), Diabetic: STZ-diabetic untreated animals (n=11), Diabetic+Va: STZ-diabetic animals treated with vanadyl sulfate (n=11). Diabetes was induced by intraperitoneal injection of STZ in a single dose of 65 mg/kg body weight. STZ was dissolved in a freshly prepared 0.01 M citrate buffer (pH 4.5). Vanadyl sulfate was given by gavage at a dose of 100 mg/kg body weight daily for 60 days. The body weight of all rats was measured at days 0, 1, 30 and 60 [12]. At the last day of the experiment (60th day), rats were sacrificed, stomach tissue was taken and homogenized by means of a glass homogenizer in cold saline to make a 10% (w/v) homogenate.

Biochemical assays

After STZ injections, blood samples of the rats were collected from the tail vein at days 0, 1, 30 and 60. Blood glucose levels after 18 h fasting were measured [21]. The enzyme activities such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), myeloperoxidase (MPO), carbonic anhydrase (CA), glucose-6-phosphate dehydrogenase (G6PD) and lactate dehydrogenase (LDH) were assayed in appropriately diluted stomach tissue homogenates. CAT activity was assayed by measuring the decomposition rate of H_2O_2 and the enzyme activity was expressed μmol H_2O_2 consumed/min/mg protein [22]. SOD activity was assayed by its ability to increase the rate of riboflavin-sensitized photo-oxidation of o-dianisidine according to the method described by Mylroie et al. [23]. The enzyme activity was calculated using bovine erythrocyte SOD as standard and expressed as unit/mg protein. GR [24] and GPx [25] activities were monitored by following the oxidation of NADPH and the enzyme activity was expressed as μmol NADPH oxidized/min/mg protein. GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate by the method of Habig and Jacoby [26]. The method is based on the determination of the rate of conjugate formation between glutathione and CDNB. The enzyme activity was expressed as μmol CDNB conjugate formed/min/mg protein. MPO activity was measured according to Hillegass et al. [27]. One unit of the enzyme activity was defined as the amount of MPO required to decompose 1 μmole of H_2O_2 in 1 min and the results were expressed as unit/mg protein. CA activity was determined using p-nitrophenyl acetate as a substrate and the enzyme activity was defined as μmol 4-nitrophenol formed/min/mg protein [28]. G6PD activity was assayed by monitoring the NADPH production at 340 nm and the enzyme activity was expressed as μmol $NADP^+$ reduced/min/mg protein [29]. LDH activity was determined by the method of Moss and Henderson [30]. In this method NADH oxidation was monitored by the decrease in absorbance at 340 nm and the enzyme activity was defined as μmol NADH oxidized/min/mg protein. Total protein content was assayed by the method of Lowry et al. [31], using bovine serum albumin as a standard.

Statistical analysis

The results were given as mean±SD and evaluated parametrically using an unpaired *t*-test and ANOVA vari-

TABLE 1. Mean levels of weight parameters (g) for all groups [12].

Group	0 Day	1 Day	30 Day	60 Day
Control	246.64±44.43	243.35±39.50	275.51±34.40	283.48±30.35
Control+Va	224.17±16.13	221.03±12.58	253.14±14.06	251.91±20.41
Diabetic	231.67±37.71	197.02±37.47	175.74±38.48	171.70±34.67
Diabetic+Va	229.65±32.14	203.93±30.57	197.46±34.40	199.16±36.20
P_{ANOVA}	0.571	0.011	0.0001	0.0001

The values were given as mean±SD.

TABLE 2. Mean levels of blood glucose (mmol/L) for all groups [21].

Group	0 Day	1 Day	30 Day	60 Day
Control	4.14±0.82	4.34±0.71	4.49±0.74	3.83±0.51
Control+Va	4.05±0.52	4.72±1.07	4.22±0.90	4.80±0.61
Diabetic	3.64±0.32	11.75±2.39	12.03±2.03	17.95±7.29
Diabetic+Va	3.99±0.76	14.09±2.14	7.54±2.56	7.31±4.01
P_{ANOVA}	0.324	0.0001	0.0001	0.0001

The values were given as mean±SD.

TABLE 3. Effect of vanadium supplementation on oxidative stress parameters in stomach tissue of STZ-diabetic rats

Group	CAT	SOD	GR	GPx	GST	MPO	CA	G6PD	LDH
Control	4.8±1.4	0.32±0.09	4.3±0.9	441.1±147.4	65.6±12.9	0.03±0.01	1.6±0.3	0.02±0.01	13.3±4.5
Control+Va	4.4±1.3	0.26±0.04	6.3±0.3	347.8±109.0	61.5±15.3	0.04±0.02	1.6±0.4	0.01±0.01	11.7±3.9
Diabetic	5.2±2.0	0.51±0.04[a]	13.5±1.4[b]	680.1±182.1[a]	89.4±23.0[a]	0.03±0.01	2.1±0.5[a]	0.05±0.01[b]	28.3±13.8[a]
Diabetic+Va	3.8±1.1	0.45±0.04	10.0±2.5[c]	450.5±107.4[c]	62.6±11.5[c]	0.03±0.01	1.7±0.5	0.04±0.01	14.3±7.5
P_{ANOVA}	0.309	0.001	0.0001	0.0001	0.008	0.925	0.149	0.0001	0.016

The values were given as mean±SD.
[a] $p < 0.05$ *versus* Control; [b] $p < 0.001$ *versus* Control; [c] $p < 0.05$ *versus* Diabetic. All enzyme activities were expressed as unit/mg protein.

ance analysis with the NCSS statistical computer package. $p < 0.05$ value was considered significant.

RESULTS

The baseline characteristics of the rats, concerning body weights (g) and fasting blood glucose levels (mmol/L) were shown in Table 1. and Table 2., respectively. The effects of vanadium on oxidative stress parameters in stomach tissue were presented in Table 3. In the diabetic group, SOD ($p < 0.05$), GR ($p < 0.001$), GPx ($p < 0.05$), GST ($p < 0.05$), CA ($p < 0.05$), G6PD ($p < 0.001$) and LDH ($p < 0.05$) activities significantly but CAT activity insignificantly increased in stomach tissue when compared to the control group. Vanadium supplementation to the diabetic rats significantly ($p < 0.05$) reduced the elevated activities of GR, GPx, GST compared with the diabetic group whereas the decreases in CAT, SOD, CA, G6PD and LDH activities were insignificant. No significant change was seen for MPO activity between the groups. It was observed that vanadyl sulfate had no significant toxic effect on stomach tissue enzymes tested as seen in the group which was treated with vanadyl sulfate alone (Control+Va) when compared to the control group.

DISCUSSION

It is believed that oxidative stress plays an important role in diabetes and that the management of this phenomenon can be important in dealing with diabetic complications. In this case, vanadyl sulfate was chosen as supplement due to its known beneficial effect on diabetes. But some toxic effects of vanadium due to its accumulation in tissues are raising some questions in view of its use as alternative therapy. The present study was undertaken in order to assess the antioxidant potential of vanadium and to see whether it has any toxic effect on stomach tissue of diabetic rats or not. In a previous study the levels of lipid peroxidation and non-enzymatic glycosylation increased whereas glutathione decreased, representing increased utilization due to oxidative stress, which were reversed by the administration of vanadyl sulfate in stomach tissue of STZ-diabetic animals [32]. In the fight against oxidative stress, SOD turns the superoxide radical to hydrogen peroxide which in turn is converted to water by CAT/GPx [33]. Thus, impairment (which could be increase or decrease) in enzyme levels is accepted as marker for oxidative stress. In the present study CAT and SOD levels increased in the stomach tissue of diabetic rats due to the need for antioxidant defense, and vanadium supplementation restored the impaired enzyme levels, showing its beneficial effect. GPx has been shown to be important in increased peroxidative stress [34]. In our study a significant increase in GPx and GR activities was found which confirms an efficacious defense of the diabetic stomach against oxidative stress. The data obtained were similar to those presented by Gumieniczek et al. [35] for the diabetic heart. In agreement with our previous study [36] vanadyl sulfate restored the increased levels of GR and GPx showing its protective effect. Similarly, impairment in CAT, SOD, GR and GPx activities in stomach of STZ-diabetic animals was recently reported [3]. GST is a group of multifunctional detoxification enzymes, and the expression of the enzyme is affected by oxidative stress, usually observed in diabetes [37]. In the

stomach tissue, the significant raise in GST levels in the diabetic group was returned to nearly normal control group levels, proving the antioxidant effect of vanadium. MPO is a hemoperoxidase released by polimorphnuclear neutrophils which catalyzes the formation of numerous ROS, thus has strong proinflammatory and pro-oxidative properties [38]. No significant change was found for MPO activity between the groups suggesting that this mechanism was not involved in this case. CA is a class of zinc metalloenzymes that reversibly catalyzes hydration of carbon dioxide to bicarbonate and a proton [39]. CA isoenzymes have also been shown to be overexpressed in the cellular response to oxidative stress [40]. In the present study, we found that there was an increase in CA activity in the stomach tissue of the diabetic rats, in accordance with our previous studies [21, 41]. Administration of vanadium provoked a decrease in CA activity, however the difference was not significant. G6PD is the principle source of NADPH which is of central importance to cellular redox regulation and any changes in G6PD will alter NADPH levels, thus impact the entire antioxidant system and makes tissues very vulnerable to oxidative damage [42]. Significantly increased activity of G6PD was observed in stomach tissue of diabetic rats, however vanadium supplemention did not alter the enzyme activity showing that vanadium does not interfere with this key enzyme of carbohydrate metabolism. According to Ainscow et al. [43] overexpression of LDH activity may be directly responsible for insulin secretory defect in some forms of diabetes. Increased LDH activity in diabetic rats has also been reported by various researchers [44-46]. Similarly, in this study an increase in the activity of LDH was observed in the stomach tissue of diabetic rats which was slightly reduced by vanadium treatment.

CONCLUSION

This study has demonstrated that administration of vanadium at a dose of 100 mg/kg body weight showed ameliorative effect against oxidative stress in the stomach tissues of STZ-diabetic rats. Thus, we suggest that this trace element could be used as antioxidant in diabetic complications.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest.

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