

Study of the beneficial effect of vanadium sulfate on the liver of experimental diabetic rats

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ABSTRACT

Background and Aims: Liver is a tissue that is negatively affected by diabetes mellitus (DM). This is due to its central function in the regulation of carbohydrate metabolism. Vanadium salts, which have insulinomimetic effects, have been found to stimulate glycogenesis and glycolysis, as well as to inhibit glycogenolysis and lipolysis. The aim of this study is to evaluate the effect of vanadium sulfate (VS) on biochemical changes in liver tissue of diabetic rats.

Methods: Randomly selected 6.0 - 6.5 months Swiss Albino rats were separated into two diabetic and two control groups. Group I: non treated animals. Group II: non treated animals orally administered VS (100mg/kg/day for 60 days), group III: STZ-induced diabetic animals (65 mg/kg with intraperitoneally) and group IV: STZ-induced diabetic animals administered VS (at same dose and time). Antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, as well as alkaline phosphatase, glucose-6-phosphate dehydrogenase, carbonic anhydrase, myeloperoxidase, paraoxonase and lactate dehydrogenase were estimated in liver tissue homogenates of the groups.

Results: Liver catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, lactate dehydrogenase, carbonic anhydrase and paraoxonase activities were decrease, but alkaline phosphatase, glucose-6-phosphate dehydrogenase and myeloperoxidase activities were increase in diabetic rats when compared to normal rats.

Conclusion: Results show that VS restored altered parameters in the liver tissue and prevented oxidative stress in diabetic rats.

Keywords: Liver, vanadium sulfate, diabetes mellitus, hyperglycaemia, antioxidant enzymes, streptozotocin, oxidative stress

INTRODUCTION

Hyperglycaemia is the primary clinical indication of diabetic manifestation and is responsible for the development of many chronic diabetic complications. It is associated with long-term microvascular and macrovascular damage, with the dysfunction and failure of various organs, leading to atherosclerosis, blindness, renal failure and neuropathy (Hussain, Claussen, Ramachandran, & Williams, 2007). Diabetic complications induced by hyperglycaemia are due to free radical generation, thus perturb cellular antioxidant defence systems and damage cells. In a typical diabetic profile, the formation of free radicals is disproportionate. Oxidation of glucose, non-enzymatic gly-

cation of proteins, increased transcription factors and oxidative degradation of glycosylated proteins are the main responsible factors for this occurrence (Pari, & Saravanan, 2007). Particularly high free radical development and weakening of the antioxidant defence mechanism can cause damage to cell organelles and enzymes, as well as lipid peroxidation and increased insulin resistance (Maritim, Sanders, & Watkins, 2003).

The liver has a central function in regulating carbohydrate metabolism by maintaining blood sugar level and the continuous supply of glucose to meet the needs of other organs. A significant part of liver damage is caused by reactive oxygen species (ROS) and β cell dysfunction, which in turn is damaged by

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autoimmune and inflammatory reactions (Molehin, & Oloyede, 2018).

Different types of insulin arrangements and synthetic medical remedies are available for clinical use in diabetic patients. Unfortunately, none of the available treatment strategies restores the release of physiological insulin, or has an effect in healing the cellular lesions caused by diabetes. As current treatments produce undesirable side effects and contraindications, the necessity of new approaches to DM treatment has arisen (Wagenaar, Kuck, & Hoekstra, 1999; Luft, Schmülling, & Eggstein, 1978). A variety of vanadium compounds have been shown to have insulinomimetic properties in animal model and cell culture systems. They exhibit great potential for the pharmacotherapy of diabetes (Yanardag, et al., & Bolkent, 2009; Zhang, Yang, Wang, & Crans, 2006).

Vanadium is an essential element which is responsible of regulation in biological systems. Just like other essential micronutrients, this ultra-trace element is required in small quantities for the cells to maintain their normal functions and growth and development of healthy organisms. It affects the activity of enzymes, adjust the second messengers actions, some signal transduction cascades and carbohydrate metabolism, mimics insulin and growth factor activities, induce protein tyrosine kinase activity, decrease phosphotyrosine phosphatases and regulate expression of genes (Chakraborty et al., 2007). Vanadium ions and its complexes exert various insulinomimetic and anti-diabetic properties, for instance increasing glucose transport in the liver tissue and skeletal muscle, stimulating the glycogen synthesis and lipogenesis as well as adipocyte metabolism, and inhibiting lipolysis and protein catabolism (Gao et al., 2006). Previous investigations indicate that vanadium compounds inhibit protein tyrosine phosphatase activity (an enzyme involved in the direct phosphorylation of insulin receptor substrate 1, stimulation of insulin receptor and cytosolic non-receptor tyrosine kinase activity, as well as the activation of phosphatidylinositol 3 kinase) leading to glucose transporter 4 (GLUT4) translocation (Kawabe, Yoshikawa, Adachi, & Sakurai, 2006).

The present study was carried out to evaluate the effect of vanadium sulfate (VS) on biochemical changes in liver tissue of diabetic rats. Our aim is to focus on the beneficial effect of VS on the oxidatively damaged liver tissue of streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Experimental rats

In this study, 6.0-6.5 month old male rats were used. All animals were kept in cages at standard conditions.

Induction of diabetes

The rats were made diabetic with a single dose of streptozotocin (STZ). STZ were (65 mg/kg) dissolved in a freshly solution (citrate buffer 0.01 M pH=4.5) intraperitoneally (Junod, Lambert, Stauffacher, & Renold, 1969). The data related with blood glucose concentrations were are presented in our previous study (Koyuturk, Tunali, Bolkent, & Yanardag, 2005).

Experimental design

The experiments were examined and confirmed by the Local Institute's Animal Care and Use Committees of Istanbul University. Four groups were randomly created; group I untreated, nondiabetic controls (n=13); group II nondiabetic control animals administered VS (n=5); group III diabetes induced animals with STZ (n=11); group IV STZ-induced diabetic animals administered with VS (n=11). VS was given (100 mg/kg/day) for 60 days without interruption by gavage technique. Blood glucose concentrations were determined according to the o-toluidine method (Relander, & Rähä, 1963).

Preparation of liver tissue homogenates

At 60 days all fasted animals were sacrificed under anesthesia. Liver tissues were taken, washed with cold solution (0.9% saline) and frozen until needed for study at -76 °C. After made 10% (w/v) homogenate with glass equipment, clear supernatants were obtained by centrifugation and used for analysis of enzymatic antioxidants, tissue enzyme activities and protein level estimation.

Assay of liver enzymes activities and protein level

Freshly prepared homogenates were used to assay for enzyme activities as follows: catalase (CAT) (Aebi, 1984), superoxide dismutase (SOD) (Mylorie, Collins, Umbles, & Kyle, 1986), glutathione peroxidase (GPx) (Paglia, & Valentine, 1967), glutathione reductase (GR) (Beutler, 1971), glutathione-S-transferase (GST) (Habig, 1981), alkaline phosphatase (ALP) (Walter, & Schult, 1974), glucose-6-phosphate dehydrogenase (G6PD) (Betke et al., 1967), lactate dehydrogenase (LDH) (Wroblewski, 1957), carbonic anhydrase (CA) (Verpoorte, 1967), myeloperoxidase (MPO) (Wei, & Frenkel, 1991) and paraoxonase (PON) (Furlong, Richter, Seidel, & Motulsky, 1988). Liver protein level was measured by the method of Lowry using bovine serum albumin as standard (Lowry, Rosebrough, Farr, & Randall, 1951).

Statistical data

The unpaired Student's t-test and analysis of variance (ANOVA) were used to analyse biochemical results calculated with statistical computer package (NCSS). Mann-Whitney test was used for comparison between control and experimental animals. The results were performed as mean \pm SD. $p < 0.05$ values were accepted as significant.

RESULTS

Table 1 shows the activities of antioxidant enzymes in all groups. A significant decrease in CAT, SOD, GPx, GR and GST activities in liver tissue of STZ-diabetic group compared to control group ($^aP < 0.0001$) was observed. The activities of the enzymes were increased in diabetic group given VS compared to non-treated diabetic group ($^bP < 0.0001$). A significant difference was observed between all groups ($P_{ANOVA} = 0.0001$; Table 1).

Liver tissue ALP, G6PDH and LDH activities were significantly increased ($^aP < 0.0001$) in diabetic rats as compared to the control group. Orally given VS was meaningfully reduced liver ALP, G6PDH and LDH activities ($^bP < 0.0001$, $^cP < 0.001$) when compared to diabetic rats. All enzymatic activities were observed to have significant difference between the groups ($P_{ANOVA} = 0.0001$; Table 2).

Table 1. Liver CAT, SOD, GPx, GR and GST activities of control and diabetic rats.*

Groups	CAT (U/mg protein)	SOD (U/mg protein)	GPx (U/gprotein)	GR (U/mg protein)	GST (U/mg protein)
Control	2733.25±65.34	2.91±0.39	3632.18±69.5	0.0232±0.0008	3.85±0.04
Control + VS	3080.93±118.86	1.82±0.32	3351.84±31.62	0.0188±0.0006	4.37±0.06
Diabetic	1526.74±50.31 ^a	1.92±0.14 ^a	2187.58±65.79 ^a	0.0130±0.0003 ^a	2.29±0.06 ^a
Diabetic + VS	2722.35±107.18 ^b	3.20±0.15 ^b	3890.72±45.92 ^b	0.0250±0.0003 ^b	3.80±0.06 ^b
P _{ANOVA}	0.0001	0.0001	0.0001	0.0001	0.0001

*Mean ± SD, ^aP < 0.0001 compared to control rats, ^bP < 0.0001 compared to diabetic rats.

Table 2. Liver ALP, G6PDH and LDH activities of control and diabetic rats*.

Groups	ALP (U/mg protein)	G6PDH (U/g protein)	LDH (U/mg protein)
Control	4.40±0.88	5.10±0.40	38.36±3.71
Control + VS	7.53±1.32	6.25±0.12	26.68±7.32
Diabetic	8.24±0.80 ^a	7.90±0.28 ^a	119.57±7.99 ^a
Diabetic + VS	4.94±0.48 ^b	5.68±0.18 ^b	87.61±13.64 ^c
P _{ANOVA}	0.0001	0.0001	0.0001

*Mean ± SD, ^aP < 0.0001 compared to control rats, ^bP < 0.0001 compared to diabetic rats, ^cP < 0.001 compared to diabetic rats.

Liver CA, MPO and PON activities of control and diabetic rats are presented in Table 3. The liver CA and PON activities at 60 days of diabetes induction showed a significant decrease when compared to control rats (^aP<0.0001). Treatment with VS significantly reversed the altered activities of CA and PON of the diabetic rats in comparison to non-treated diabetic animals (^bP < 0.0001). On the other hand, experimentally-induced diabetes caused a mean increase in liver MPO activity (^aP<0.0001) in comparison to control animals. Orally vanadyl treated diabetic group demonstrated significant decrease in MPO activity when compared with STZ-induced diabetic group (^bP<0.0001). A significant difference was observed between treated and non-treated animals (P_{ANOVA}= 0.0001; Table 3).

Table 3. Liver CA, MPO and PON activities of control and diabetic rats.*

Groups	CA (U/mg protein)	MPO (U/g protein)	PON (U/g protein)
Control	7.58±1.42	0.41±0.09	21.22±2.44
Control + VS	4.00±0.94	0.57±0.01	20.38±1.06
Diabetic	3.98±0.50 ^a	0.60±0.01 ^a	15.93±0.55 ^a
Diabetic + VS	6.96±0.86 ^b	0.26±0.03 ^b	19.12±1.50 ^b
P _{ANOVA}	0.0001	0.0001	0.0001

*Mean ± SD, ^aP < 0.0001 compared to control rats, ^bP < 0.0001 compared to diabetic rats.

DISCUSSION

Oxidative stress, resulting from the production of uncontrolled reactive oxygen species (ROS) is a condition that is attributed and accepted in the aetiology of diabetes. Alterations in the antioxidant enzymes activities makes tissues defenceless to oxidative stress and this leads to the onset and progression of diabetes complications (Pandey & Rizvi, 2009; Lipinski, 2001).

Increased ROS production due to persistent hyperglycaemia may lead to a decrease in hepatic antioxidant enzyme activities and depletion of hepatic antioxidants. Treatment with VS significantly improved CAT, SOD and GSH related enzymatic activities (GPx, GR and GST) in hepatic system. The observed results may be attributable to the antioxidant activity of VS.

It was found in our previous work that VS treatment alleviated some liver tissue alterations such as decreased level of reduced glutathione (GSH) that are associated with diabetic state (Koyuturk et al., 2005). This could indicate an increase in activities of antioxidant enzymes of the liver tissue, suggests the normal functioning and protective activity of vanadium and supports the hepatoprotective efficacy of VS.

ALP is an enzyme indicator of hepatic lesion and a biomarker for acute hepatotoxicity in type 1 DM (Rodríguez, Plavnik, & Tolosa de Talamoni, 2018; Kanikarla-Marie & Jain, 2015; Liang et al., 2015). The increased activity of liver ALP activity suggest defective utilization of glucose. The administration of VS in our study normalized the ALP activity in the diabetic liver tissue.

The fate of glucose through the pentose phosphate pathway has not been well characterized, more so there is conflicting evidence about the activity of G6PDH in DM. Gupte et al., (2009) demonstrated that the expression of protein level and activity of G6PDH is much higher in the liver in the type 2 DM Zucker obese rats (Gupte et al., 2009). Accordingly, in the present study, we observed an increased G6PDH activity in diabetic liver tissue. The higher G6PDH activities drive NADPH - a fuel of NADPH oxidase, and O₂⁻ production. The studies suggest that this is one of the reasons of increased oxidative stress in multi-organ dysfunction and damage in experimental diabetic rats (Gupte et al., 2009; Gupte et al., 2005; Matsui et al., 2005). In our study, oral vanadium supplementation decreased significantly the activity of this enzyme in hepatic tissue, thus highlighting another possible mechanism of anti diabetogenic activity.

The lactic acid formation from pyruvate is catalysed by LDH, a cytosolic enzyme in anaerobic glycolysis pathway. In hyperglycemic state, a significant increase in LDH activity was found due to impaired glucose-induced insulin secretion in diabetes (Ainscow, Zhao & Rutter, 2000). The LDH system reflects NAD⁺/NADH ratio indicated by the lactate/pyruvate ratio of the hepatocyte cytosol (Sekar, Sivagnanam & Subramanian, 2005). Similarly, in the present study, a significant increase in LDH activity of was observed in the diabetic group. A significant reduction in the LDH activity was observed due to the regulation of the ratio of NAD⁺/NADH by oxidation of blood glucose, in VS given diabetic rats.

CA is involved in many important physiological and pathological conditions, and provides HCO₃⁻ as a substrate for pyruvate carboxylase (Ismail, 2018). It is reported that the concentration of CA-III is reduced in the liver and serum of STZ-induced DM adult male rats (Nishita, Igarashi, & Asari, 1995). In our study, oral VS treatment increased the CA activity in diabetic rats. This increase may lead to increased hepatic glucose production by the presence of these substrates.

Increased MPO production in neutrophilic granulocytes can produce strong oxidants (such as HOCl, HOBr), provoke oxidation of nitric oxide and nitration of tyrosine. This MPO-derived reactive species could eventually mediate the development of oxidative damages in liver tissue. Wiersma et al., (2008) suggested that type 2 DM is associated with mildly increased plasma levels of MPO. In our research, we observed a significant increase in MPO activity in the diabetic tissue. Antioxidant effect of vanadium decreased MPO activity and this suggest a reduction in oxidative stress and subsequent neutrophil production.

PON1 plays an important role in the regulation of glucose metabolism. In the muscles, GLUT4 expression is upregulated by PON1 in an insulin receptor-independent manner (Koren-Gluzer, Aviram & Hayek, 2013). Besides that, PON1 regulates directly some important enzymes of glycolytic pathway, (Meneses, Silvestre, Sousa-Lima, & Macedo, 2019; Koren-Gluzer et al., 2013; Khersonsky & Tawfik, 2006). Our data showed that VS significantly increased the activity of PON, an enzyme mainly synthesized in the liver tissue.

CONCLUSION

In conclusion, the findings of the current study demonstrate that VS may provide effective protection against oxidative damage in liver tissue of STZ-induced diabetic rats. This suggests that vanadium is able to ameliorate the enzymatic antioxidant defence systems and prevent peroxidation in tissue. VS significantly improved metabolic alterations, oxidative and inflammatory status in liver tissue through its antidiabetic, anti-oxidative and anti-inflammatory effects. Therefore, VS could be an ideal auxiliary treatment option for DM.

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S.T., R.Y.; Final Approval and Accountability- S.T., A.P., İ.A., R.Y.; Technical or Material Support- S.T., A.P.; Supervision- R.Y.

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