Vanadyl Sulfate, Taurine, and Combined Vanadyl Sulfate and Taurine Treatments in Diabetic Rats: Effects on the Oxidative and Antioxidative Systems

Sibel Tas, Emre Sarandol, Sedif Ziyonok Ayvalik, Zehra Serdar, and Melahat Dirican

Department of Biology, Science and Literature Faculty and Department of Biochemistry, Medical Faculty, Uludag University, Bursa, Turkey

Received for publication June 14, 2006; accepted September 18, 2006 (ARCMED-D-06-00247).

Background. Vanadyl sulfate (VS) and taurine are two promising agents in the treatment of diabetes related to their antihyperglycemic, antihyperlipidemic, and hyperinsulinemic effects. Data about the effects of VS on the oxidant—antioxidant system is limited and controversial. However, taurine is a well-documented antioxidant agent and our aim was to investigate the effects of VS, taurine and VS and taurine combination on the oxidative—antioxidative systems in streptozotocin—nicotinamide (STZ-NA) diabetic rats.

Methods. Nicotinamide (230 mg/kg, i.p.) and streptozotocin (65 mg/kg, i.p.) were administered. VS (0.75 mg/mL) and taurine (1%) were added to drinking water for 5 weeks. Rats were divided as control (C), diabetes (D), diabetes + VS (D + VS), diabetes + taurine (D + T), diabetes + VS and taurine (D + VST). Plasma and tissue malondialdehyde (MDA) levels were measured by high-performance liquid chromatography and spectrophotometry, respectively. Paraoxonase and arylesterase activities were measured by spectrophotometric methods and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined using commercial kits.

Results. VS, taurine and VS and taurine combination treatments reduced the enhanced blood glucose, serum total cholesterol and triglyceride, tissue MDA and plasma MDA (except in the D + VS group) levels and increased the reduced serum insulin level, serum paraoxonase and arylesterase activities, GSH-Px activity and SOD activity (except in the D + VS group).

Conclusions. The findings of the present study suggest that VS and taurine exert beneficial effects on the blood glucose and lipid levels in STZ-NA diabetic rats. However, VS might exert prooxidative or antioxidative effects in various components of the body and taurine and VS combination might be an alternative for sole VS administration. © 2007 IMSS. Published by Elsevier Inc.

Key Words: Streptozotocin, Nicotinamide, Oxidative stress, Paraoxonase, Vanadyl sulfate, Taurine.

Introduction

Oxidative stress is the imbalance between oxidant and antioxidant systems in favor of the former. Clinical and experimental studies have shown that oxidative stress is involved in the pathogenesis of many diabetic complications (1–3). Hyperglycemia is blamed for the complications of diabetes because elevated glucose concentration directly injures cells and induces lipid peroxidation (2). Furthermore, antioxidative enzyme activities might also be reduced due to glycation or increased lipid peroxidation products (1,4–6). Hyperlipidemia, which is a common feature of diabetes, is also suggested to be involved in the increased lipid peroxidation (1–3). Several investigators suggested...
that treatment of hyperglycemia and hyperlipidemia may be beneficial in diminishing oxidative stress and related complications in diabetes mellitus.

Vanadyl sulfate (VS) and taurine (2-amino ethane sulfonic acid) are two promising agents in the treatment of diabetes mellitus. Vanadium complexes improve beta-cell insulin store and secretory function and can reverse the diabetic state (7–10). Several studies have indicated that taurine supplementation improves insulin sensitivity and increases insulin activity and decreases blood lipids (11–14). The amino acid decreases visceral fat, hyperlipidemia, postprandial glucose oxidation, liver cholesterol and hyperglycemia in diabetes (15,16). Taurine is also a well-documented antioxidant agent. The antioxidative effects of taurine have been attributed to its ability to stabilize biomembranes, to scavenge reactive oxygen species, and to reduce the peroxidation of unsaturated membrane lipids (12,17–20). In addition, taurine scavenges hypochlorous acid produced by activation of granulocytes, forming taurine-chloramine; in addition, it may act as an indirect antioxidant (11,20). Data about the effects of VS on the oxidant–antioxidant balance are limited and controversial (21–26). Vanadium is a catalytic metal and, as reported in some studies, it is possible that VS induces lipid peroxidation (21–23). In other studies, authors suggested that VS exerts antioxidative effects (24–26). So the attitude towards the usage of VS is ambiguous. Some investigators suggested that combination therapies might be required to minimize the potential toxic effects of VS (27,28).

In the present study, our aim was to investigate the effects of VS, taurine and VS and taurine combination on the oxidative and the antioxidative systems in streptozotocin–nicotinamide diabetic rats. To the best of our knowledge, this is the first study investigating effects of VS and taurine combination on the oxidative–antioxidative system in diabetes mellitus. For this purpose, we determined serum paraoxonase and arylesterase activities, serum total antioxidant capacity (TAOC), erythrocyte superoxide dismutase (SOD) and blood glutathione peroxidase (GSH-Px) activities to evaluate the antioxidative mechanisms and, to evaluate lipid peroxidation status, we measured malondialdehyde (MDA) levels in plasma and in tissues (Musculus gastrocnemius, heart, liver, kidney) of the streptozotocin–nicotinamide (STZ-NA)-induced diabetic rats.

**Materials and Methods**

**Animals**

The experiments were performed with 40 male Wistar strain rats weighing approximately 350–400 g. Rats were given free access to standard laboratory chow (carbohydrates 35%, proteins 25%, lipids 7%, and vitamins 3%) and tap water for 1 week before the experiment. Based on water intake, the mean daily intake of taurine was 2.80 and 2.20 g/kg body weight in D + T and D + VST groups, respectively. The mean daily intake of VS was 1.19 and 0.91 mg/kg body weight D + VS and D + VST groups respectively. Four rats were housed per cage. The study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of Uludag University, Bursa.

**Experimental Design**

Rats were divided into five groups of eight rats each: Group I: Normal control rats (C); Group II: STZ-NA induced diabetic rats (D); Group III: Diabetic rats with orally administered VS (D + VS); Group IV: Diabetic rats with orally administered taurine (D + T); Group V: Diabetic rats with orally administered vanadyl sulfate and taurine (D + VST).

**Diabetes Induction**

Streptozotocin–nicotinamide diabetes was induced by i.p. injection of 230 mg/kg of nicotinamide (Sigma, St. Louis, MO) dissolved in saline, 15 min before an i.p. injection of 65 mg/kg STZ (Sigma), freshly dissolved in sodium citrate buffer (pH 4.5) (29). Control rats received an injection of citrate buffer alone. Rats with blood glucose levels ≥11.1 mmol/L were considered as diabetic and were used for the study.

**Vanadyl Sulfate Treatment**

Vanadyl sulfate (Sigma-Aldrich) was given in drinking water on the seventh day following STZ injection at a concentration of 0.5 mg/mL for the first 3 days and then 0.75 mg/mL during 5 weeks of the treatment period (groups III and V). The concentrations of VS were chosen based on a previous report (30) that demonstrated a pronounced glucose-lowering effect in diabetic rats.

**Taurine Treatment**

After the seventh day following STZ injection, rats in groups IV and group V were given taurine (Sigma-Aldrich) 1% in drinking water for 5 weeks. Each day, when fresh water (with or without VS or T) was provided, the volume of liquid consumed during the previous 24 h was recorded.

**Sample Preparation**

At the end of the experimental periods, blood samples were obtained by cardiac puncture under light ether anesthesia following 10–12 h of fasting. Liver, kidney, heart and skeletal muscle (Musculus gastrocnemius) tissues were removed immediately after blood collection, rinsed with cold saline, blotted with gauze and stored at −20°C until
analysis. Blood samples were drawn in heparin-coated, EDTA-containing and non-additive tubes. Serum aliquots for TAOC measurements were kept at −70°C until the analyses were performed. A part of whole blood was frozen for GSH-Px determination. Erythrocytes for SOD determination were washed by saline and frozen after hemolysis.

**Analytes**

Blood glucose concentration was measured with a glucostix strip test in a glucometer (Abbot Glucometer Medisense Products, Abbott Park, IL). Serum insulin level was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). Total cholesterol (TC), triglyceride (TG) and HDL-cholesterol (HDL-C) were determined with the aid of enzymatic test kits (Randox Laboratories, Antrim, UK).

Paraoxonase activity was determined as described by Eckerson et al. (31). The rate of hydrolysis of paraoxon was measured by monitoring the increase in absorbance at 412 nm at 25°C. Paraoxonase activity is expressed in U/L serum and defined as 1 μmol p-nitrophenol generated per minute under the above conditions.

Arylesterase activity was determined by using phenylacetate as the substrate. The reaction mixture contained 1.0 mM phenylacetate and 0.9 mM calcium chloride in 9.0 mM Tris-HCl buffer, pH 8.0. One unit of arylesterase activity is defined as 1 μmol phenol generated per minute under the above conditions and expressed as kU/L serum (32).

Erythrocyte SOD and GSH-Px activities were determined using Randox kits (Randox Laboratories). Briefly, the determination of SOD activity was based on the production of O₂− anions by the xanthine/xanthine oxidase system (33). GSH-Px was catalyzed by the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The generation of nicotinamide adenine dinucleotide phosphate was measured spectrophotometrically at 340 nm. The activity of GSH-Px was expressed as U/mL.

TAOC was measured in serum by means of a commercial kit (Randox Laboratories). The assay is based on the incubation of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS⁺, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAOC measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L).

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA (34). Plasma MDA concentrations were determined with the high-performance liquid chromatography (HPLC) procedure of Young and Trimble (35). A calibration curve was prepared for each day by using 1,1′,3,3′ tetraethoxypropane as the standard.

**Statistical Analysis**

Statistical analyses were carried out by SPSS 10 program for Windows (SPSS, Chicago, IL). Data are presented as mean ± SEM. For statistical analysis, Kruskal-Wallis test was used, followed by the Mann-Whitney U test. A level of p <0.05 was accepted as statistically significant.

**Results**

Blood glucose levels and body weights of the study groups were recorded each week and are shown in Figure 1. Blood glucose, TC and TG levels were significantly increased, whereas body weight and insulin levels were significantly decreased in the D group compared with the C group. Glucose levels were decreased and body weight and insulin levels were increased in the D + VS, D + T and D + VST groups compared with the D group. A significant reduction in serum TC and TG levels in the D + VS, D + T and D + VST groups were observed compared with the D group (Table 1). HDL-C levels were significantly decreased in the D group compared with the C group. Glucose levels were decreased and body weight and insulin levels were increased in the D + VS, D + T and D + VST groups compared with the D group. A significant reduction in serum TC and TG levels in the D + VS, D + T and D + VST groups were observed compared with the D group (Table 1). HDL-C levels were significantly decreased in the D group compared with the C group.

![Figure 1](image-url) Blood glucose level (A) and body weight (B) records of the study groups.
Reduced oxidants were significantly lower in the D and D + VST groups compared with the D + VS group (Table 2). Serum paraoxonase and arylesterase activities were significantly higher in the D + VS group compared with the C group and the D + VST groups compared with the D + VS group (Table 1).

Table 1. Serum insulin, total cholesterol (TC) triglyceride (TG) and high-density lipoprotein-cholesterol (HDL-C) levels in control and experimental groups of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>D</th>
<th>D + VS</th>
<th>D + T</th>
<th>D + VST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>7.4 ± 0.3</td>
<td>16.2 ± 0.3**</td>
<td>11.9 ± 1.2**</td>
<td>12.4 ± 1.4**</td>
<td>10.9 ± 1.4**</td>
</tr>
<tr>
<td>Insulin (mIU/mL)</td>
<td>31.0 ± 0.4</td>
<td>22.8 ± 1.6**</td>
<td>42.4 ± 2.4**</td>
<td>49.7 ± 6.0**</td>
<td>47.2 ± 4.3**</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>3.19 ± 0.25</td>
<td>4.26 ± 0.15**</td>
<td>2.98 ± 0.13**</td>
<td>3.16 ± 0.13**</td>
<td>3.01 ± 0.09**</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.25 ± 0.15</td>
<td>4.97 ± 0.65**</td>
<td>2.10 ± 0.35**</td>
<td>0.77 ± 0.05**</td>
<td>0.65 ± 0.07**</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.20 ± 0.49</td>
<td>0.96 ± 0.14**</td>
<td>0.97 ± 0.16</td>
<td>1.22 ± 0.17</td>
<td>1.21 ± 0.15**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM for eight rats in each group.

Discussion

We observed oxidative stress in STZ-NA-induced diabetic rats, which is reflected by increased plasma and tissue MDA levels and this finding is in line with the previous reports (36–38). Hyperglycemia and hyperlipidemia, as demonstrated in this study, might be associated with the oxidative stress (1–3). SOD and GSH-Px activities were significantly decreased in all tissue MDA levels compared with the D group (Figure 2). Plasma and heart and liver tissue MDA levels were significantly lower in the D + VS group compared with the D + VST groups and these results are in line with the previous reports (36–38). Hyperglycemia and hyperlipidemia, as demonstrated in this study, might be associated with the oxidative stress (1–3). SOD and GSH-Px activities were significantly lower in the D group compared with the C group and the D + VST groups compared with the D + VS group.
reported as decreased, unchanged and increased in type 2 diabetes (39–43), and we found increased GSH-Px and SOD activities in diabetic rats. The elevation in SOD and GSH-Px activities might be a protective mechanism against oxidative stress. Paraoxonase (PON1), an esterase, is located on HDL and protects both HDL and LDL from oxidation (4–6). PON1 exerts paraoxonase and arylesterase activities, since the enzyme hydrolyzes organophosphates (such as paraoxon) and aromatic esters (such as phenyl acetate) (4–6,44,45). Arylesterase activity born by PON1 is suggested to be an index of actual protein concentration (44). There is evidence that low serum PON1 or arylesterase activity is associated with several risk factors for coronary heart disease, including diabetes and hypercholesterolemia. In the present study serum paraoxonase and arylesterase activities were significantly reduced in the STZ-NA diabetic rats, which is consistent with previous human studies (4–6,45). Decreased serum paraoxonase and arylesterase activities could be related to oxidative stress and hyperglycemia since the enzyme activities might be inhibited by glycation or increased lipid peroxidation products (5,6,45). Reduction in the enzyme protein synthesis, which was reflected by decreased serum arylesterase activity could be the reason or a contributing factor

**Figure 2.** Malondialdehyde (MDA) levels in plasma (nmol/mL) and tissues (nmol/mg tissue) of the control and experimental rats. Values are expressed as mean ± SEM for eight rats in each group. Statistical comparison: "C vs. D,"D vs. D + VS or D + T or D + VST, "D + VS vs. D + T or D + VST, "D + T vs. D + VST. Statistical significance, *p <0.05; **p <0.01.
for the decreased paraoxonase activity (45). Since PON1 is an HDL-associated enzyme, decreased HDL levels may result in reduced serum paraoxonase activity. In the present study, HDL-standardized enzyme activity (PON1/HDL-C ratio) was determined to assess whether the altered paraoxonase activity was due to the HDL-C level reduction. Although serum paraoxonase activity was significantly reduced in the D group, there were no significant differences in the ratios of paraoxonase activity to HDL-C levels between the control and the D groups. This finding might suggest that the decreased serum paraoxonase activity might be related to the decreased HDL-C levels observed in the D group.

In the present study, we found that insulin level increased and blood glucose and lipids decreased in the D + VS, D + T and D + VST groups compared with the D group. These results suggest that agents alone or in combination acted as an insulin secretagogue. STZ-NA-induced diabetes is a model that mimics some features of type 2 diabetes and is characterized with a partial responsiveness to glucose (42). As a model, it has some limitations for type 2 diabetes, which is characterized with a decreased responsiveness to glucose. STZ-NA-induced diabetes is an insulin secretagogue. STZ-NA-induced diabetes is a model that mimics some features of type 2 diabetes and is characterized with a partial responsiveness to glucose (42). As a model, it has some limitations for type 2 diabetes, which is characterized with a decreased responsiveness to glucose. STZ-NA-induced diabetes is an insulin secretagogue. STZ-NA-induced diabetes is a model that mimics some features of type 2 diabetes and is characterized with a partial responsiveness to glucose (42). As a model, it has some limitations for type 2 diabetes, which is characterized with a decreased responsiveness to glucose.

There is limited and conflicting knowledge about the effects of vanadium compounds on the oxidant—antioxidant systems in diabetes mellitus (21–26). In the present study, tissue MDA levels were significantly reduced; however, plasma MDA levels were significantly increased in the D + VS group compared to those of the D group. Plasma lipoproteins and the tissues are the main sources of plasma MDA. The increased plasma MDA levels seem to be related to the oxidation of lipoproteins since tissue MDA levels were significantly reduced in the D + VS group. In line with our results, Lapenna et al. (21) reported that VS increased the oxidizability of lipoproteins in patients with type 1 and 2 diabetes. However, Goldfine et al. (46) reported that 6 weeks of VS administration to type 2 diabetic patients did not alter serum MDA levels. Parallel to our results, Genet et al. (47) reported that tissue MDA levels were reduced in orthovanadate-treated type 1 diabetic rats. However, Thompson and McNeill (48) reported increased MDA levels in VS-treated diabetic and control hepatic cells, but not in red blood cells; and Preet et al. (24) found that the increased MDA levels in the lens of the diabetic rats were not altered in response to vanadium treatment. VS was shown to accumulate in different concentrations in various tissues and the plasma (22,49,50), and its effects might be concentration dependent (28,49–51) so the discrepancy in MDA levels might partly be related to the different effects of the VS in the plasma and the tissues.

Serum paraoxonase and arylesterase and whole blood GSH-Px activities were significantly increased and erythrocyte SOD activity was unchanged in the D + VS group compared with those of the D group. In line with our results, BolKent et al. (10) reported increased blood glutathione levels in the blood of VS-treated type 1 diabetic rats. Increment in these antioxidant enzyme activities might be related to hypoglycemic effect of VS. Additionally, increased serum paraoxonase activity might be related to increased synthesis of the enzyme protein, as reflected by the increased arylesterase activity.

Both plasma and tissue MDA levels were significantly reduced in the D + T group compared to those of the D group. These alterations might be related to hypolipidemic, hypoglycemic (as demonstrated in the present study) and direct antioxidative effects of taurine. The antioxidative and hypoglycemic effects of taurine might also be involved in the changes in antioxidative enzyme activities. Furthermore, taurine might also indirectly induce GSH-Px activities. Since taurine is synthesized from cysteine, which is a precursor of GSH (52), taurine supplementation might bring about enhancement in GSH levels by directing cysteine into the GSH synthesis pathway (52,53). Serum paraoxonase and arylesterase activities were also increased in the D + T, which might also be related to hypoglycemic and antioxidative effects of taurine. As mentioned above, increased paraoxonase activity might be related to increased synthesis of the enzyme protein. Furthermore, HDL-standardized enzyme activity was significantly higher than in the D group, which suggested that the increased paraoxonase activity was related to alterations in HDL-C levels. The beneficial effect of taurine was also reflected by increased TAOC observed in the D + T group.

The findings of the D + VST group were similar to those of the D + T group. Both plasma and tissue MDA levels were significantly lower and antioxidative enzyme activities and TAOC were higher in the D + VST group compared to those of the D group.

When we compared the VS and VST groups with the T group (since taurine is a well-documented antioxidant), we observed that VS showed profound increase in the MDA levels of the kidney and muscle tissues and VST combination showed in the kidney, liver and heart tissues. Kidney is one of the most common targets for diabetic complications. Therefore, we suggested that VS treatment might be critical for better protection of some tissues from oxidative stress. However, the possible prooxidative action of VS should be taken into account.

The findings of the present study suggest that VS, taurine and VS and taurine treatments exert beneficial effects on the blood glucose and lipid levels in STZ-NA diabetic rats. Furthermore, these three treatment modalities reduced oxidative stress with one exceptional effect observed (plasma MDA level) in the VS-treated diabetic rats. This finding supported the hypothesis that VS might exert prooxidative or antioxidative effects in various components of the body and the suggestion that combination therapies might be required to minimize the potential toxic effects of VS. We suggest further studies investigating effects of VS and...
taurine and other antioxidants in various combinations and different dosages.

Acknowledgments
This study was supported by a grant from Uludag University Research Foundation (2002/51).

References