Abstract

The objective of the study was to assess the effects of both 5-vanadium organic complexes with bipyridine and phenanthroline (1-5) and small insulin injections on oxidative stress indices in streptozotocin (STZ)-induced diabetes in rats (Di1-Di5). In all groups of the animals, the glucose, uric acid, urea, α−tocopherol, and SOD activity were measured in the plasma, and the GSH level was measured in the liver. The glucose level was higher in all the diabetic (D) rats; however, no differences were observed in the vanadium-treated STZ rats. Of all the STZ-treated rats, the highest level of uric acid was observed in the diabetic control group (CD). Substantial differences in the uric acid level were observed between the diabetic control group and some of the vanadium-treated groups: Di1, Di4, and Di5. Significant differences for the urea level were observed between all the diabetic rats and healthy rats. For the α−tocopherol level, a difference was observed only between the Di and Di3 groups. A difference in SOD activity was observed between the Di and Di4 groups. The type of vanadium complex also had an influence on SOD activity, where differences were observed in the Di3 group in contrast to the Di4 and Di5 groups. In three groups of vanadium-treated STZ diabetic rats (Di1, Di2, and Di4) the glutathione level in the liver was higher than in the STZ-diabetic rats, as well as in the normal control rats. In conclusion, the type of administered complexes of vanadium and diabetes had different influences on the antioxidant defence.

Key words: rats, oxidative stress, streptozotocin, diabetes.

Vanadium is an essential element for all living organisms. Numerous studies have proved its essential role in biochemical mechanisms, especially in the case of azotobacter (9, 24), ascidians (18, 34), and mushrooms (11). During the biochemical processes in these organisms, the vanadium ion alters the levels of oxidation. Studies have shown that in mammals, vanadium has more functions. Vanadium influences the inhibition and activation of enzymes (14). Several studies have shown the anti-diabetic activity of vanadium in both types of diabetes (29, 33). Other studies have demonstrated positive vanadium influence on anti-HIV (36) and in the prevention of carcinogens in vitro (20).

Vanadium in high doses can be toxic for people where they are exposed to this metal in industry or in individual accidental cases. The aforementioned element does not occur in significant amounts in natural food (4, 26). On the other hand, some types of drinks, for example, beer and wine, have a high level of vanadium, sometimes over 100 µg/L (32). The absorption of vanadium from the intestinal system in normal conditions is lower than 20% of an administered dose (2, 10). A lack of vanadium has never been observed in people. However, it has been observed in rats, chickens, and goats (21). During its path through the intestines and its diffusion into the blood, the oxidative state of vanadium can change depending on the type of compound administered. The transformation of the oxidative state in organisms is associated with different types of mechanisms. Glutathione, catechol, cysteine, NADH, NADPH, and L-ascorbic acid play a great role in the abovementioned transformation. In blood, vanadium (V) is dominant, but vanadium (IV) dominates inside cells. Ions V⁵⁺ and V⁴⁺ can pair with ADP, ATP, aminoacids such as glutamin acid, cysteine, glicine, peptides, and proteins (27, 35, 38). A number of experiments have proven the presence of vanadium (III) inside cells. In living organisms, transferrin, ferritine, lactoferine, and calmodulin (8, 16) can bind the vanadium and create complexes, which transport this metal to the tissues. The quantity of vanadium in administered doses influences the total oxidative state in the living organism. Several experiments on the activity of anti-oxidative enzymes have shown diverse responses in different organs to higher-than-normal vanadium doses (27, 28, 31, 35, 38). The aim of this experiment was to study the influence of the administration of new
vanadium complexes on the oxidative defence system in streptozotocin-induced diabetes in rats, with doses of insulin injection lower (1U/kg) than usually used.

**Material and Methods**

**Synthesis of vanadium complexes.**

1. Bisperoxo complexes of vanadium (V) of Na[VO(O₂)₂L] • n H₂O type (complexes 1-3).

   Ten millimole of NaVO₃ was dissolved in molar excess of 10% H₂O₂ (the molar ratio of H₂O₂ to vanadium is 1:3). Twenty millititre of ethanolic solution containing 10 mmol of polypyridine (L) was added drop-wise, with constant stirring, to the resulting clear, yellow solution, cooled in an ice bath. The temperature of the reaction mixture did not exceed 10°C during the synthesis. Subsequently, 50 ml of cooled ethanol was added to precipitate yellow crystals. The solid phase was filtered off in a funnel with a glass frit, and washed with 10 ml of cold ethanol. The complexes were dried with air in a dark place for 24 h, then collected and stored in a refrigerator.

2. Vanadyl complexes of [VO(SO₄)L] • n H₂O type (complexes 4 and 5).

   An equimolar hot solution of polypyridines (L) in ethanol (30 ml) was added drop-wise, while stirring the reaction mixture, to a hot solution of VOSO₄ • 5 H₂O (5 mmol) in ethanol (30 ml), containing the minimal amount of water to dissolve VOSO₄. The solution was cooled to room temperature, and concentrated if required. The green precipitate, which appeared was filtered off, washed with ethanol and ethyl ether, and dried in vacuo. The synthesis was carried out under an argon atmosphere in order to prevent the oxidation of the vanadium (IV).

   The purity of the complexes was confirmed by microanalysis and IR spectroscopy. The synthesised complexes are shown in Table 1. The structure of the ligands and their complexes with vanadium is shown in Fig. 1.

**Animals and vanadium administration.** Male Wistar rats weighing 220-250 g were adapted for 12/12 h day/night cycle; night was from 10 am to 10 pm with humidity ranging between 75%-85 %. The animals were divided into eight groups of six animals (three animals in each cage). Three days later, 55 mg of STZ in a citric buffer (0.1 mol/L) solution per 1 kg of b.w. was injected into the caudal vein in a volume of 1 mL/kg of b.w. in seven groups of animals. The eighth group of animals was the control group. Three days subsequent to the injections, the glucose level was measured with an Exac Tech (Medisense) strip glucometer. Each rat had a glucose level higher than 17 mmol/L. Consequently, the rats were separated into tested groups: diabetic control rats (DC), diabetic rats with insulin (Di), five groups of diabetic rats with insulin and tested vanadium complexes (Di 1-5, e.g. group Di3 represents diabetic rats to which both insulin and complex number 3 were administered simultaneously), and one group of normal control rats (C). The complexes were administered once a day before 10 a.m. by gavage in a dose of 50 µmol/kg b.w., and 1 U/kg of insulin was injected subcutaneously at the same time.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Formula</th>
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<tr>
<td>1</td>
<td>Na[(O₂)₂(2,2'-bpy)] • 8 H₂O</td>
</tr>
<tr>
<td>2</td>
<td>Na[VO(O₂)₂(1,10'-phen)] • 5 H₂O</td>
</tr>
<tr>
<td>3</td>
<td>Na[VO(O₂)₂(4,4'-Me₂-2,2'-bpy)] • 8 H₂O</td>
</tr>
<tr>
<td>4</td>
<td>[VO(SO₄)(1,10'-phen)] • 2 H₂O</td>
</tr>
<tr>
<td>5</td>
<td>[VO(SO₄)(2,2'-bpy)] • H₂O</td>
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**Table 1. Vanadium complexes with polypyridines**

**Fig. 1. Structure of the complexes with polypyridines**
Eight weeks after beginning the administration of the complexes, the rats were anaesthetised (thiopental 50 mg/kg b.w.), and the blood was collected into a vessel with heparin, and the kidneys, heart, pancreas, liver, and spleen were collected. The blood was centrifuged to separate plasma from the blood cells. The organs were frozen in –20°C for further analysis.

Biochemical analysis. Glucose, uric acid, and urea levels in the plasma were measured using a Konelab 30 analyser in a biochemical laboratory.

The α-tocopherol was determined in accordance with the HPLC method described earlier (12). Briefly, fresh plasma was mixed with ethanol containing butylated hydroxytoluene (BHT), ascorbic acid, pyrogallol, and racemic tocotol as an internal standard. The tocopherol and tocol were extracted into the hexane by vortex mixing for 1 min. The hexane phase was evaporated, and the residue was re-dissolved in the mobile phase; 97:3 (v/v) n-hexane:2-propanol. HPLC with fluorimetric detection was performed at the wavelength of 298 ex/325 em. The data was expressed as μg of α tocopherol per ml of plasma.

SOD activity. 0.1 ml of plasma was put into a spectrophotometric cuvette containing carbonate buffer (0.005 mol, pH 10.2), EDTA (1*10⁻⁴ mol), and epinephrine (3*10⁻⁵ mol). The oxidation of epinephrine to adrenochrome was measured over 4 min by the increase of absorbance in 480 nm (19). One unit was defined as the amount of enzyme required for a 50% inhibition in the epinephrine auto-oxidation. The activities were expressed as U/mL.

Reduced glutathione. The samples of the liver (2 g) were homogenised with an ice-cold buffer (0.01 M Tris/HCl, pH 7.4). The homogenate was centrifuged for 20 min at 10,000 g at 4°C. In the supernatant, the reduced level of glutathione was measured using Ellman’s spectrophotometric method (25). The data was expressed as glutathion mmol/g of wet liver.

Statistical analysis. All statistic calculations were made using the Statistica 7.1 programme. Differences between the groups were calculated by the nonparametric Kruskal-Wallis test.

Results and Discussion

Numerous articles have informed us about the potential role of vanadium in both types of diabetes treatment. Vanadium in mammalian organisms usually has two oxidative states: (IV and V). The possibility of changes in the oxidative states by vanadium can cause positive and negative effects on the organism. The direction of this variation was strongly associated with certain parameters of the antioxidant defence and the vanadium complex and dose of this compound. The pancreas of STZ-treated rats produced very little insulin. This deficiency resulted in a high concentration of glucose in the blood. Several days after the STZ injection, numerous biochemical changes were observed in the living organism. An increase in the production of free radicals was also associated with a high glucose level after the degradation of β-cells in the pancreas by STZ injection. The study presented shows the results of our investigation on different vanadium organic complexes in STZ diabetic rats. Usually, vanadium compounds are administered in higher doses – 0.1-0.7 mmol/kg (7) - than in the study presented, where the tested complexes were administered once daily by gavage in doses lower than the usual 50 μmol/kg, with insulin (ultralente) injected daily in small doses. A small dose of vanadium complexes was administered to prevent the toxic, distinctive effects of high doses (21). The authors wanted to verify the hypothesis that the simultaneous administration of small doses of insulin and vanadium can show positive synergistic effects on the glucose tolerance and antioxidant status in STZ rats.

This relationship could be useful in the treatment of diabetes, since such synergistic mechanism is frequently used in the treatment of cardiovascular diseases. A similar experiment conducted by Goldfine et al. (13) proved that the administration of vanadium allowed diabetic patients to decrease their daily insulin dose.

The glucose level in all rats injected with STZ was statistically higher than in the healthy control rats, and we did not observe any differences in any of the STZ rats. (Fig. 2)

However, we did not observe any decrease in the glucose level in the insulin and vanadium-treated rats injected with STZ. This result could be linked to the methodology of the study. The last dose of insulin and vanadium was administered 24 h before the blood collection, which may be the reason for the increase in the glucose level after a period of decreasing. To confirm this supposition, the percentage of HbA1c (T1/2 3 months) or glucose level profile has to be assayed in future studies at several different periods between the administration of these two drugs. Indirectly, we can come to some conclusions regarding this action from the biochemical parameters of these rats.

Rats from the vanadium-treated diabetic groups had visible changes in the uric acid, urea, and tocopherol levels, contrary to the diabetic control rats. Uric acid, urea, α–tocopherol, SOD, and glutathione are some of the most important factors in anti-oxidative defence (1, 3, 17, 30, 37). In our work, the changes in the level of uric acid between the vanadium-treated and non-treated diabetic rats are presented as well.

Usually, diabetes increases the uric acid level in living organisms (15). This observation was confirmed in this study, where the uric acid level in the diabetic control group was significantly higher than in the healthy control rats (Fig. 3). The vanadium complexes (1, 4 and 5) significantly influenced the normalisation of this parameter. A similar observation was made by Yanardag et al. (38), who proved that the administration of vanadyl sulfate significantly decreased the uric acid level in the healthy control group. During the last 10 years, the influence of vanadium on the uric acid level has only been researched by Yanardag et al. (38).
<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose [mmol/l]</th>
<th>Uric acid [mmol/l]</th>
<th>Urea [mmol/l]</th>
<th>α-tocopherol</th>
<th>GSH mmol/g liver</th>
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<tr>
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<td>8</td>
<td>1</td>
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<td>16</td>
<td>18</td>
<td>6</td>
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**Fig. 2.** Plasma level of glucose (Di1, Di2, Di3, Di4, Di5, Di, CD vs C, P<0.05).

**Fig. 3.** Plasma level of uric acid (CD vs Di1, Di4, Di5 and C, P<0.05).

**Fig. 4.** Plasma level of urea (Di1, Di2, Di3, Di4, Di5, CD and Di vs C, P<0.05).

**Fig. 5.** Plasma level of α-tocopherol (Di3 vs Di, P<0.05).

**Fig. 6.** SOD activity in plasma (Di3 vs Di4 and Di5, P<0.05, and for Di4, Di5, CD vs C, P<0.05).

**Fig. 7.** GSH in the liver (Di2, Di3, Di5 and CD vs Di, P<0.05, and for Di1, Di2, Di4 and Di vs C, P<0.05).
The urea level in the study by Ramachadran et al. (23) was statistically lower in the vanadium-treated diabetic rats. In our study, the level of urea in the vanadium-treated diabetic rats was higher than in the non-treated rats and lower than in the healthy rats but no statistical significance of this parameter was observed (Fig. 4). This could be the result of the different types of complexes of vanadium or administered doses: 5 mg/kg b.w./ml/d in Ramachadran’s study.

Vitamin E has antioxidant properties. A decrease in this vitamin’s level is associated with a high glucose level (6, 22). Changes in α-tocopherol levels were not significant (Fig. 5). Considerable changes were observed only between the Di3 and Di groups. Generally, the available literature does not contain any information on the influence of vanadium administration on the tocopherol level, and therefore it is very difficult to interpret the results of our experiment. Unaided tocopherol was used as a supplement in diabetes with beneficial effects on glycaemic control and insulin resistance, but other studies have reported no effects (7).

SOD activity was different, depending on the type of vanadium complex administered. Furthermore, changes in the group not treated with vanadium were observed, where the SOD activity was higher than in the control diabetes group (Fig. 6). The aforementioned observation differed from the one made by Tas et al. (31), who observed a higher SOD activity in vanadium-treated rats. These results can be associated with the type of vanadium compounds administered. They administered inorganic vanadium sulfate. Our vanadium compounds have different organic ligands and oxidative states (IV and V). Perhaps these ligands can influence SOD plasma activity. This observation was confirmed by Soares et al. (28), who proved that SOD activity differed significantly in mitochondria in Sparus aurata cardiac tissue, after decap- and metavanadate administration. In the present study, vanadium (IV) complexes demonstrated an inhibitory effect on SOD activity in comparison to the insulin-injected diabetic group (Di), but the effect was not statistically significant. Vanadium (V) peroxocomplexes had no effect on SOD activity.

Glutathione changes were more significant than other measured parameters (Fig. 7). The changes were probably the result of the influence of the insulin and the type of vanadium complex administered. The glutathione level in the healthy rats and the diabetic rats was almost identical, but it was significantly higher in the group with insulin administration. Our results differed from the work of Bolkent et al. (5) group, where the glutathione level in the control group was significantly higher than in the diabetic group. The increase in the glutathione level in diabetic rats with insulin injections is exceptionally interesting. Furthermore, all vanadium-treated rats had a higher glutathione level in comparison to the diabetic group.

Because of the lack of scientific studies dealing with this subject in a similar manner, it is impossible to conduct a broader interpretation of the data obtained. Further research is required in order to reveal the nature of the mechanisms resulting in figures demonstrated.

In conclusion, small doses of vanadium complexes and insulin administered simultaneously to rats with STZ-induced diabetes did not drastically affect the anti-oxidative defence system. It is suggested that vanadium in 50 µmol/kg doses can be used without any toxicological effects. Occasionally, it is possible to observe a different effect in the uric acid level, SOD activity, and GSH level, in comparison to the oxidative state of the vanadium and ligands. In order to gain better recognition of the vanadium complexes’ influence on oxidative defence, it is necessary to conduct further research with higher doses of vanadium.

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References


