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EFFECTS OF VANADIUM COMPLEXES SUPPLEMENTATION ON V, Cu, Mn, K, Fe, Zn, AND Ca CONCENTRATION IN STZ DIABETIC RATS PANCREAS

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Abstract: The objective of the study was to assess the effects of $Na[V^{V}O(O_2)_2(2,2'\text{-bpy})] \times 8\ H_2O$ (complex 1), $Na[V^{V}O(O_2)_2(1,10'\text{-phen})] \times 5\ H_2O$ (complex 2), $Na[V^{V}O(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \times 8\ H_2O$ (complex 3), $[V^{IV}O(SO_4)(1,10'\text{-phen})] \times 2\ H_2O$, (complex 4), $[V^{IV}O(SO_4)(2,2'\text{-bpy})] \times H_2O$ (complex 5), where: 2,2'-bpy = 2,2'-bipyridine, 1,10'-phen = 1,10'-phenanthroline, 4,4'-Me-2,2'-bpy = 4,4'-dimethyl-2,2'-bipyridine and a small insulin injection on V, Cu, Mn, K, Fe, Zn, and Ca concentration in the STZ (streptozotocin) diabetic rats pancreas during a 5-week treatment with the tested complexes. In all groups of animals metal concentration in the pancreas was investigated by means of Proton Induced X-ray Emission (PIXE) method. Maximum concentration of vanadium was observed in the pancreas for complex 5 (1.69 ± 0.09 mg/kg dry weight), lower for complex 3 (1.51 ± 0.10 mg/kg dry weight), and the lowest for complex 1 (1.21 ± 0.27 mg/kg dry weight) supplementation. The influence of vanadium administration on other metals' concentration in the rats' pancreas was also investigated. All vanadium-tested complexes showed an increase of zinc concentration in the examined pancreas in comparison to the diabetic animals not treated with vanadium. The results were the highest for complex 1 and the lowest for complex 5. The concentration of Fe, Cu, Mn, K and Ca in the pancreas is not evidently influenced by administration of the vanadium complexes.

Keywords: pancreas, rats, PIXE, diabetes, vanadium, copper, manganese, potassium, iron, zinc, calcium

Diabetes, especially type 2, affects increasing numbers of people and creates a global threat to health and life (1). According to the World Health Organization, after the year 2020, the number of patients suffering from diabetes will be doubled. In this disease the irregular distribution of carbonate, resulting from the insulin deficiency in case of Type 1 diabetes and from insulin resistance in case of Type 2 diabetes is a characteristic event. The cause for this injury related to hyperglycemia is the formation of glycated proteins, glucose oxidation, and increased level of free fatty acids (2). This results in oxidative stress in the cells, as well as activation of oxidative and inflammatory signalling pathways, which continue to damage the insulin-diarrhoea producing cells, what leads eventually to various complications of diabetes.

At present, the basic aim of the research is to identify and understand the exact mechanisms of the development of diabetes. The pancreas is a large gland located behind the stomach and next to the gallbladder. It produces digestive enzymes and hormones, including insulin and glucagon that help to regulate blood's sugar levels. It was also shown that the levels of indicators of oxidative stress firstly increase and then decrease after antioxidants supplementation (2). This suggests that chronic pancreatitis (CP) must involve a state of heightened free rad-

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ical mediated injury, and that damage apparently can be inversed in many people with antioxidant supplements. CP is a progressive inflammatory disease, that can lead to a loss of the function of the pancreas. As a result, people with CP can lose too much weight, suffer frequent diarrhoea, and development of diabetes or vitamin deficiencies. Patients with exocrine pancreatic insufficiency are at a greater risk of developing trace-element deficiencies as a result of malabsorption than patients with exocrine pancreatic sufficiency (1–3).

Dietary supplementation with micronutrients may be a complement to classical therapies for preventing and treating diabetic complications. Supplementation is expected to be more effective when a deficiency in these micronutrients exists. Some vitamins, such as vitamins E, C, and A and the carotenoids as well as trace elements such as selenium and zinc, have antioxidant properties (4). In diabetes, cells and tissues are damaged due to the imbalance between the production and the removal of free radicals. The effective biologic antioxidants for oxidative stress, such as α-lipoic acid, vitamin E and selenium, are effective in diminishing oxidative damage like membrane lipid peroxidation (5). However, zinc is involved in a multitude of these processes within the pancreas, including glucagon secretion, digestive enzyme activity, and insulin packaging, secretion and signalling. As a result of this extensive physiological contribution, deregulations of Zn metabolism within the pancreas impairs a multitude of key processes, including glycemic control (6, 7).

The prevalence of lower plasma concentrations of trace elements in patients with CP has been reported (5). Diabetes mellitus is also associated with deficiencies of vitamins and trace elements, particularly those of vitamin C (9–11), vitamin E (12) and zinc (5, 13, 14). The concentration of glucose is the most important physiological factor regulating the secretion of insulin in the blood flowing from pancreatic β cells.

The concentration of glucose in the blood influences both the secretion and the biosynthesis of that hormone. Simultaneously, insulin increases the activity of proteins transporting glucose (GLUT4) into the cell. This is connected with the glucose membrane receptor stimulation, which in turn affects the increase of the ATP amount in those cells. Numerous investigations of the activity of the vanadium complexes have shown promising results with regard to their potential use in medical care (15–17).

Vanadium is an element, which occurs in living organisms in trace amounts but it may still influence the metabolism of carbohydrates, lipids and cholesterol. Nevertheless, the exceeded level of this element is toxic. Anti-cancer/anti-carcinogen property of this element has also been noted (18).

Tolman et al. (19) demonstrated insulin-mimetic properties of the vanadium salts *in vitro*. This study has shown that various vanadium salts, similarly as insulin, stimulate the transportation and oxidation of glucose in adipocytes, increase the glycogen synthesis in the rat diaphragm and hepatocytes, and inhibit gluconeogenesis (GNG). The research conducted by Heyliger et al. (20) demonstrated that as a result of sodium vanadate treatment in the animal diabetes model, the normalization of the glucose level in the hyperglycemia conditions was achieved. It led to further research on the possibility of application of various vanadium compounds in the treatment of people with the Type 1 or Type 2 diabetes (21, 22).

Vanadium concentration in the blood of mammals is about 0.2–0.5 ng/mL, out of which nearly 80% is present in plasma, as a component of proteins such as transferrin and albumins (23). As the role of vanadium has not been explained yet, it seems justified to study the impact of vanadium compounds on oxidative stress.

Vanadium ions in body fluids are mainly present in the form of pentavalent metavanadium, while the compounds of this element at the fourth oxidation level show intra-cellular domination.

Vanadium ions enter the cell *via* the anion canals, where their reduction takes place, primarily in the presence of glutathione, catechol, cysteine, NADH, NADPH and L-ascorbic acid (24).

MATERIALS AND METHODS

Material and methods of the experiment were described in earlier publications (25, 26).

Animals and vanadium administration

Male Wistar rats weighing between 220–250 g were adapted to a 12 h/12 h day/night cycle, (day was from 8 a.m. to 8 p.m.) with humidity ranging between 75–85%. The animals were divided into 7 groups of 6 animals in each group. The animals from each group were housed in two cages (3 rats in each). After 3 days from the beginning of the experiment, 55 mg of streptozotocin in citric buffer (0.1 mol/L) solution per 1 kg of body mass was injected into the caudal vein in the volume of 1 mL/kg of body mass in all groups of animals. Three days subsequent to the injections, the level of glucose was measured using Exac Tech (Medisense) strip glu-

cometer. The glucose level measured in animal blood was higher than 17 mmol/L. After the measurement, the rats were separated into the following tested groups, diabetic control rats (D), diabetic rats treated with insulin (Di), 5 groups of diabetic rats treated with both insulin and tested complexes (Di 1–5). The water solutions of tested vanadium complexes were administered once a day before 10 a.m. by gavage in a dose of 50 μmol/kg and 1 U/kg of insulin was injected subcutaneously. Five weeks after the beginning of the the treatment, the rats were anesthetized (using thiopental 50 mg/kg) and then, the pancreas was collected. The organ was kept frozen in –20°C until the time of the analysis.

Synthesis of vanadium complexes

The complexes $Na[V^{V}O(O_2)_2(2,2'\text{-bpy})] \times 8\ H_2O$ (complex 1), $Na[V^{V}O(O_2)_2(1,10'\text{-phen})] \times 5\ H_2O$ (complex 2), $Na[V^{V}O(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \times 8\ H_2O$ (complex 3), $[V^{IV}O(SO_4)(1,10'\text{-phen})] \times 2\ H_2O$, (complex 4), $[V^{IV}O(SO_4)(2,2'\text{-bpy})] \times H_2O$ (complex 5), where: 2,2'-bpy = 2,2'-bipyridine, 1,10'-phen = 1,10'-phenanthroline, 4,4'-Me-2,2'-bpy = 4,4'-dimethyl-2,2'-bipyridine were synthesized using methods described in the literature and their purity was confirmed by microanalysis and IR spectroscopy (27–29).

Pancreas tissue preparation

Frozen pancreas tissue was transferred directly to the lyophilizing cabinet and lyophilized using ABCONO FREEZONE 4,5. in the temperature from –40°C to –53°C, and under pressure of 14 Pa to 1 Pa. Then, the lyophilized organs were homogenized and pressed into pellets, about 1 mm thickness and 10 mm in diameter, under the pressure of 15 MPa. Such pellets were placed on Scotch tape and attached to an aluminum frame.

PIXE analysis

The PIXE (Proton Induced X-ray Emission) analysis was performed at the Institute of Nuclear Physics Polish Academy of Sciences (IFJ PAN) in Kraków (30). A multi-elemental probing was carried out using a 2 MeV proton beam (about 0.5 mm in diameter) from the Van de Graaff accelerator directed perpendicularly to the sample's surface. In order to get high quality X-ray characteristic spectra, the acquisition time for measuring each sample was set to 20 min. In every series 14 samples together with 2 standards (IAEA H-8 Horse Kidney and National Standards & Technology Standard Reference Material 1577b Bovine Liver) were mounted in the PIXE chamber. The standard materials were used for energy calibration and determination of the trace elements' concentration levels. All the emitted X-ray quanta were detected with Si-Li detector with the energy resolution of 190 eV for the 5.9 keV line. The normalization was performed based on simultaneously detected spectra of back-scattered protons. Both the X-rays and back-scattered protons were recorded using Computer Automated Measurement and Control (CAMAC) electronic system. All the acquired spectra were analyzed with GupixWin ver. 2.0 software. All results, showing the level of metals' concentration in the rats' pancreas, are presented as 'box-and-whisker' plots showing median, lower and upper quartiles (box) and the farthest data (whiskers). The statistical calculations were performed using Statistica 7.1 program. Differences between the studied rat groups were estimated with the use of a non-parametric Kruskal-Wallis test that enables to compare three or more unpaired groups. Results with p level below 0.05 were considered statistically significant ($p < 0.05$).

RESULTS

Vanadium

The level of vanadium in pancreas of diabetic not-treated rats (D) and insulin-injected diabetic ones (Di) was in the range from 0.25 ± 0.06 to 0.33 ± 0.05 mg/kg of dry tissue. Similar data were reported by Frank et al. (31). In pancreas of vanadium compounds treated rats (Di1–Di5) the concentration of this element increased about 6 times as compared to the diabetic control groups D and about 4–5 times as compared to Di group, regardless of the chemical structure of the compound and oxidation state of vanadium (Fig. 1). This observation is similar to the data presented by Cremer et al. (32) where the rats were treated with an ^{48}V isotope complex. The total absorption of vanadium after oral administration of the tested complexes was not calculated, however, it is widely known that the level of V measured in intestinal system equals roughly to ten percent of an administered dose, and such a level of vanadium uptake was assumed in the presented study (33). The vanadium concentration in pancreas of vanadium-treated rats' ranged from 1.21 ± 0.27 to 1.69 ± 0.09 mg/kg of dry tissue. The lowest concentration growth of vanadium was observed in the pancreas of animals treated with complex 4, and the largest – with complex 5. Both of the compounds have the same vanadium oxidation state (IV) but different ligands. The changes of the vanadium content in pancreas of rats supplemented with compound 5

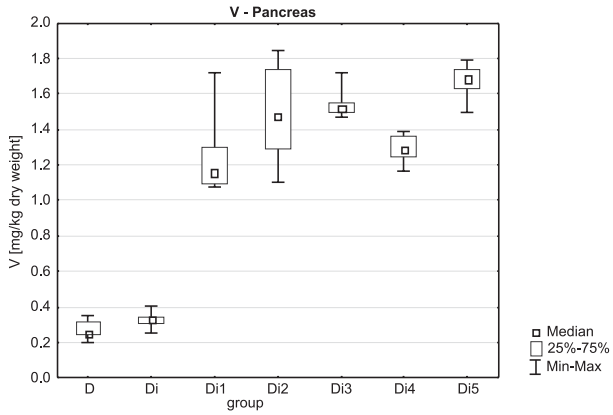

Figure 1. Vanadium level in the study rat groups: (diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1- $Na[VO(O_2)_2(2,2'\text{-bpy})] \cdot 8\ H_2O$, Di2- $Na[VO(O_2)_2(1,10'\text{-phen})] \cdot 5\ H_2O$, Di3- $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \cdot 8\ H_2O$, Di4- $[VO(SO_4)(1,10'\text{-phen})] \cdot 2\ H_2O$, Di5- $[VO(SO_4)(2,2'\text{-bpy})] \cdot H_2O$)

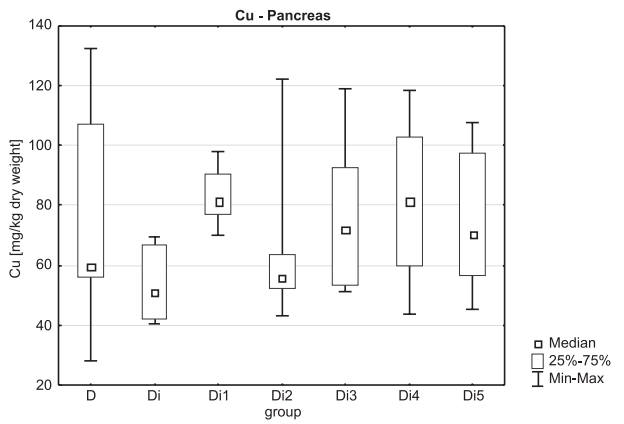

Figure 2. Copper level in animal groups: (diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1- $Na[VO(O_2)_2(2,2'\text{-bpy})] \cdot 8\ H_2O$, Di2- $Na[VO(O_2)_2(1,10'\text{-phen})] \cdot 5\ H_2O$, Di3- $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \cdot 8\ H_2O$, Di4- $[VO(SO_4)(1,10'\text{-phen})] \cdot 2\ H_2O$, Di5- $[VO(SO_4)(2,2'\text{-bpy})] \cdot H_2O$)

were statistically significantly different in comparison to group D and Di ($p < 0.05$).

It is known that the presence of metal administration can influence the level of other metals such as iron, copper, zinc, manganese, calcium or potassium (34). Therefore, the concentrations of these metals in the pancreas have been determined in our analysis.

Copper

The contents of copper determined in pancreas for all the tested groups are presented in Fig. 2. The application of insulin (group Di) caused the largest decrease in the level of copper (50.72 ± 12.63 mg/ kg of dry tissue). Applying vanadium complexes increased the Cu level in comparison to the Di group. Statistically significant growth of the concentration of this element occurred for the complex 1, including the vanadium (V) and ligands bipyridine (Di 1), and the complex 4 (Di 4) with vanadium (IV) and ligands phenyls (82.50 ± 11.03 and 81.34 ± 26.95 mg/ kg of dry tissue, respectively).

Manganese

In case of the animals fed with the addition of vanadium complexes 1, 4 and 5, the concentration of manganese in the pancreas was reduced in comparison to the quantity of this microelement in the groups D and Di (Fig. 3). The twofold decrease of the manganese concentration was observed only in

case of the complex 1 ($p < 0.05$), while complexes 4 and 5 reduced the Mn quantity imperceptibly. The influence of complexes 2 and 3 on the Mn concentration in the pancreas was not observed.

Potassium

The concentration of potassium in the pancreas of the rats (Fig. 4), which were treated with vanadium complex 3 (16.32 ± 5.06 g/kg) increased in comparison to the animals from the group D (10.16 ± 3.27 g/kg) and Di (11.19 ± 2.17 g/kg), and the increase was statistically significant ($p < 0.05$). Organic complex of vanadium including methylbipyridine derivative (complex 3) raised the level of potassium in the pancreas in comparison to the complex including only bipyridine as a ligand (complex 1), and the result was statistically significant ($p < 0.05$).

The degree of the vanadium oxidation state essentially influenced the concentration of potassium in the pancreas. In case of vanadium complexes at the IV oxidation level (complex 4 and 5), a decrease in the potassium concentration in the pancreas was observed. The lowest potassium concentration occurred in group Di 4 (7.16 ± 1.88 g/kg). A decrease in potassium concentration in the pancreas in case of the Di 4 and Di5 groups in comparison to D and Di group, was observed but it was not statistically significant.

Iron

The highest concentration of the iron in the pancreas was observed in the diabetic control group (317.0 ± 112.9 mg/kg of dry tissue) (Fig. 5). After the injection of insulin in the Di group, the concen-

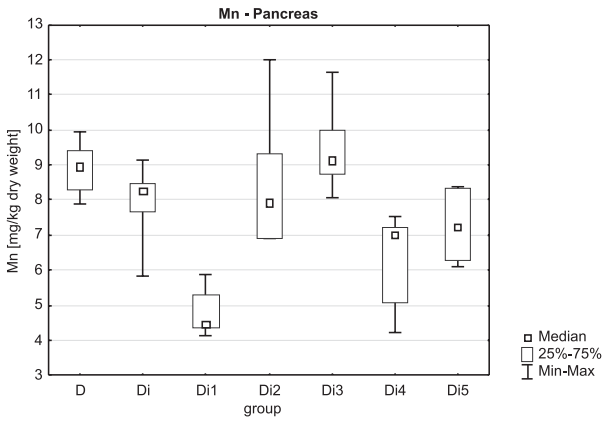

Figure 3. Manganese level in animal groups: (diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1- $Na[VO(O_2)_2(2,2'\text{-bpy})] \cdot 8\ H_2O$, Di2- $Na[VO(O_2)_2(1,10'\text{-phen})] \cdot 5\ H_2O$, Di3- $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \cdot 8\ H_2O$, Di4- $[VO(SO_4)(1,10'\text{-phen})] \cdot 2\ H_2O$, Di5- $[VO(SO_4)(2,2'\text{-bpy})] \cdot H_2O$)

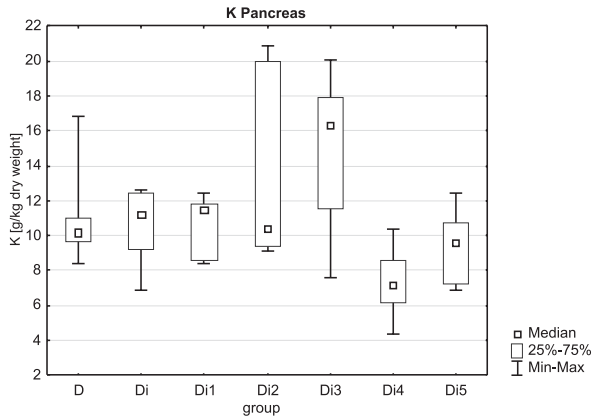

Figure 4. Potassium level in animal groups: (diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1- $Na[VO(O_2)_2(2,2'\text{-bpy})] \cdot 8\ H_2O$, Di2- $Na[VO(O_2)_2(1,10'\text{-phen})] \cdot 5\ H_2O$, Di3- $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \cdot 8\ H_2O$, Di4- $[VO(SO_4)(1,10'\text{-phen})] \cdot 2\ H_2O$, Di5- $[VO(SO_4)(2,2'\text{-bpy})] \cdot H_2O$)

tration of this element dropped twice as compared to the D group. Vanadium complexes 1, 3, 4 and 5 reduced the concentration of Fe to a statistically significant degree in comparison to the groups Di ($p < 0.05$) and D ($p < 0.05$). It was also observed that complexes 2 and 3 had not influenced the change of the concentration of Fe in comparison to the Di group.

Zinc

As shown in Fig. 6, zinc concentration was the lowest in the D control group (19.83 ± 3.28 mg/kg), not treated with vanadium. In the diabetic group with insulin administration (Di), Zn concentration was also very low (22.06 ± 33.47 mg/kg). In all the vanadium-treated animal groups, the Zn concentration increased and the level was dependent on the type of vanadium complexes used. It was statistically significant and 2 up to 6 times higher in comparison to the diabetic group D and Di. Zn concentration was the highest in the pancreas of Di 1 and Di 2 animal groups (110.77 ± 13.74 mg/kg and 84.67 ± 16.34 mg/kg, respectively). In the Di 5 group, the increase of Zn concentration was the lowest for vanadium-treated rats (41.08 ± 17.28 mg/kg) but still about two times higher in comparison to the diabetic groups (D and Di), not treated with vanadium. After both vanadium compounds 3 and 4 administration similar zinc concentration in the pancreas (64.59 ± 8.66 mg/kg and 63.36 ± 8.42 mg/kg, respectively) was observed.

Calcium

As shown in Fig 7, calcium concentration in the pancreas was higher in the animals treated by the vanadium complexes 3 and 5 (Di 3 and Di 5, respec-

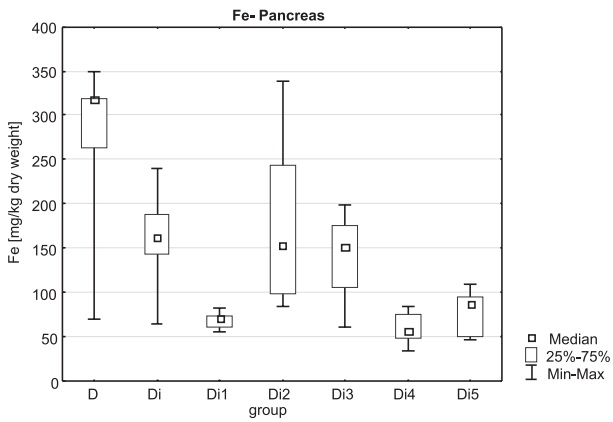

Figure 5. Iron level in the study animal groups: (diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1- $Na[VO(O_2)_2(2,2'\text{-bpy})] \cdot 8\ H_2O$, Di2- $Na[VO(O_2)_2(1,10'\text{-phen})] \cdot 5\ H_2O$, Di3- $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \cdot 8\ H_2O$, Di4- $[VO(SO_4)(1,10'\text{-phen})] \cdot 2\ H_2O$, Di5- $[VO(SO_4)(2,2'\text{-bpy})] \cdot H_2O$)

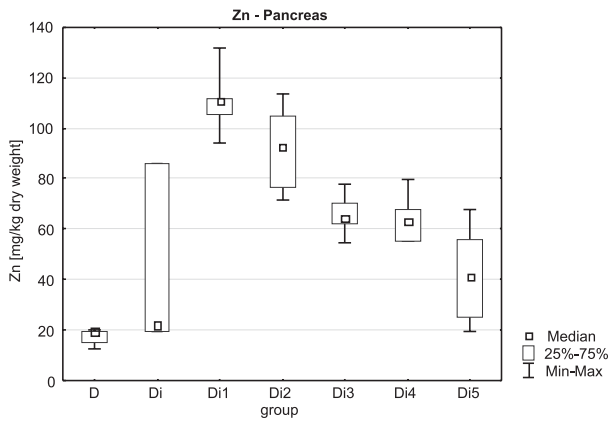

Figure 6. Zinc level in animal groups: (diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1- $Na[VO(O_2)_2(2,2'\text{-bpy})] \cdot 8\ H_2O$, Di2- $Na[VO(O_2)_2(1,10'\text{-phen})] \cdot 5\ H_2O$, Di3- $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \cdot 8\ H_2O$, Di4- $[VO(SO_4)(1,10'\text{-phen})] \cdot 2\ H_2O$, Di5- $[VO(SO_4)(2,2'\text{-bpy})] \cdot H_2O$)

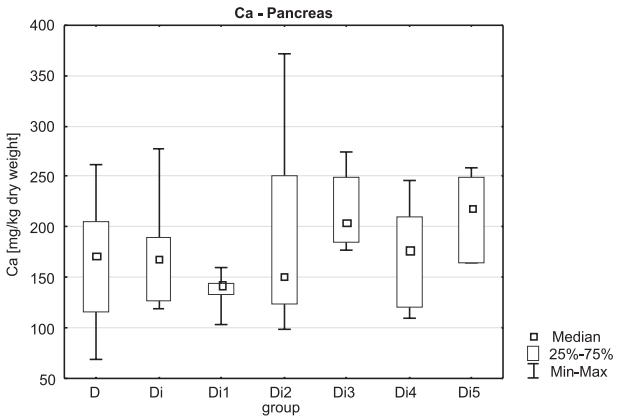

Figure 7. Calcium level in animal groups: (diabetic control rats (D), diabetic rats with insulin (Di), diabetic rats with insulin and tested complexes (Di 1- $Na[VO(O_2)_2(2,2'\text{-bpy})] \cdot 8\ H_2O$, Di2- $Na[VO(O_2)_2(1,10'\text{-phen})] \cdot 5\ H_2O$, Di3- $Na[VO(O_2)_2(4,4'\text{-Me-}2,2'\text{-bpy})] \cdot 8\ H_2O$, Di4- $[VO(SO_4)(1,10'\text{-phen})] \cdot 2\ H_2O$, Di5- $[VO(SO_4)(2,2'\text{-bpy})] \cdot H_2O$)

tively, 204.3 ± 177.8 mg/kg, 219.2 ± 39/6 mg/kg, 210.5 mg/kg) than in the D group of diabetic rats (172.3 ± 74.8 mg/kg), not treated with vanadium, and the Di group (168.3 ± 57.7 mg/kg). Vanadium (V) with bipyridine ligand (Di 1) treatment reduced calcium concentration in the pancreas in a statistically significant way in comparison to the effect of complex 3 and 5 and without statistical significance in comparison to the pancreas of not diabetic rats not treated with vanadium. Changes in the group Di 4 with vanadium (IV) with phenyl ligand in comparison to the diabetic not treated animals were not observed.

Complex 1 in comparison to the others vanadium complexes had the strongest influence on the Cu, Mn and Zn concentration and also an essential effect on Fe concentration in the pancreas and at the same time it had the weakest influence on the calcium, and potassium concentration. Complex 2 showed a strong influence on zinc concentration and mostly non influence on other elements' concentration as compared to the Di group, however a decrease in iron concentration as compared to the D group was visible. Complex 3 had a substantial influence on zinc and potassium concentration in the pancreas. All the three tested complexes (groups Di1–Di3) contained vanadium (V) and different ligands. The influence on the measured metal concentration in pancreas is associated not only with the vanadium oxidation state but also with the type of used ligands. Complexes 4 and 5 contained both vanadium (IV). Complex 4 caused more significant decrease on the potassium and iron concentrations while complex 5 on zinc concentration in the pancreas.

DISCUSSION

It has been shown that vanadium possesses anti-diabetic activity and that it can be used as a potential therapeutic agent in diabetes treatment in several diabetic models (23). Simultaneously, supplementation of this metal can influence the levels of other metals in tissues and also indirectly, metabolic parameters' such as enzymatic activity (35). Scientific investigations showed that vanadium has insulin mimetic properties and the ability of enhance the effect of insulin (22, 35). Moreover, vanadium can inhibit degradation of ligand-receptor complex in lysosomes in different types of cells (36).

Manganese is necessary for the metabolism of vitamin B1 and vitamin E. It activates some enzymes, takes part in the process of energy production, synthesis of glycogen as well as urea. During vanadium treatment, the changes of manganese concentration in rats' pancreas were not significant. This may suggest a minor influence of the tested vanadium complexes on the functions regulated by manganese in the pancreas. On the other hand, the ratio of manganese to other elements concentrations can affect the activity of some enzymes in pancreas. Therefore, the influence of vanadium on manganese level in pancreas and its possible effects should be further studied. Enzymes activated by manganese in pancreas and also possibly in other organs can be useful in the elimination of oxidative stress in patients with diabetes.

The increased potassium concentration may suggest hyperkalemia, which is usually the result of handicapped dismissing of potassium with urine and

excessive freeing of potassium from cells. This observation is associated only with the vanadium complex 3 with vanadium (V) and methylbipyridine ligand.

Iron is a component of organic biomolecules like: porphyrin, hemoglobin and myoglobin. It also occurs in active sites of some enzymes. The concentration of iron in the serum depends on absorption in the alimentary line. The highest concentration of iron is found in the intestines, the spleen (23) and the bone marrow. The decrease of the iron concentration causes, among others, a chronic failure of kidneys and/or the shortage of C vitamin absorption. In diabetic rats, not treated with insulin, the iron concentration was the highest. The administration of insulin decreases the concentration of that element in pancreas. This also confirms the insulin-mimetic action of vanadium in diabetes. The untreated diabetes and biochemical changes associated with this illness probably increase the iron concentration in pancreas tissue. This observation needs to be confirmed by additional investigation. Administration of insulin and/or vanadium caused the decrease of the copper level in pancreas. The tested vanadium complexes showed a similar influence on the level of iron in pancreas. However, the influence differed depending on the type of the vanadium complex used. In groups Di 1, Di 4 and Di 5 in comparison to the Di 2 and Di 3 the decrease of iron level was greater. Surprisingly, there's no clear trend of such influence. Complex 1 as compared to 4 and 5 differed in oxidation state (V *vs*. IV) while complex 4 differed in ligand environment as compared to complex 1 and 5. Additionally, the less significant decrease in Fe concentration in group Di2 and Di3 could be related to vanadium oxidation state (V) but this observation is in opposite to the influence of complex 1, which was also vanadium (V) component.

At the molecular and cellular level, zinc (Zn) is intimately involved in the insulin synthesis, secretion and signalling, and thus, in the subsequent effect of insulin on metabolism. Various clinical and epidemiological studies suggest that reduced Zn status is associated with diabetes (37, 38). The results of presented study support this observation and statement. The induced diabetes reduced zinc concentration in the pancreas of not treated animals.

Pancreas is the site of high Zn turnover and one of the few organs that show reduced Zn concentration during Zn deficiency (39). Reduced pancreatic Zn concentrations have been reported in genetic mouse models of Type 2 diabetes, ob/ob (mutation in ob (leptin) gene and db/db (mutation in leptin receptor) mice (40–42) and GK rats (Type 2 model produced by selective breeding of rats with glucose intolerance) (43) as compared to the non-diabetic ones.

Although a lot of effort has been placed on studying Zn and pancreas, the consequences of reduced pancreatic concentrations of other minerals (e.g., Fe, Mn and Mg in GK rats) in co-action with Zn have not been explored (42). Adequate levels of pancreatic Zn may also be crucial to provide antioxidant protection, given that oxidative stress is a factor of tissue damage in Type 1 and Type 2 diabetes and it is associated with complications occurring in this disease (44, 45). Compared to several other tissues, β-cells have lower levels of antioxidant defense components and are susceptible to oxidative damage (46–48). Zinc contributes to antioxidant defense as a component of CuZn superoxide dismutase (CuZnSOD) and metallothionein (MT).

Calcium level in different investigated groups has not changed statistically and it is difficult to discuss the mutual relationship between the diabetes insulin and the tested vanadium complexes.

This investigation showed that diabetes and its' treatment has an influence on the elemental level measured in pancreas. Pancreas, being very important organ in the diabetes control, is responsible for the glucose level changes by insulin secretion response. The investigated changes in the trace elements' level were mostly perceptible for zinc and iron. Zinc plays an important role in the physiological function of pancreas. The animal group with diabetes induced by STZ had significantly lowered zinc level in comparison to the vanadium-treated diabetic groups. Vanadium stimulates the activity of adenylate cyclase (CA), phospholipase C (PLC) and phospholipase A_2 (PLA_2) (47). This action results in the increased concentration of the secondary transmitters, such as cAMP, IP_3, DAG and also arachidic acid. The increase of IP_3 and arachidic acid level caused an increase of the level of Ca^{2+} ions. It is possible that all these mechanisms participate also in the zinc level elevation in pancreas. All the effects associated with an anti-diabetic activity of vanadium are not only the results of the insulin mimetic signalling pathway but also of the zinc concentration protection, especially in pancreas. Zinc is an element, which is known to have a protective activity in the Type 2 diabetes. Such an observation is reported for the first time in the literature. Therefore, it is not possible to discuss it with the results of other researchers.

Currently, the mutual interaction between vanadium and zinc in pancreas remains unknown. Better understanding of their interaction may offer

another possibility of increasing the understanding of diabetes mechanisms and possible procedures for treatment of that disease.

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