Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 2 pp. 271–278, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG BIOCHEMISTRY

INVESTIGATION OF THE INFLUENCE OF VANADIUM COMPOUNDS TREATMENT IN NZO MICE MODEL – PRELIMINARY STUDY

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Abstract: New Zealand obese mice (NZO) are characterized by symptoms similar to human metabolic syndrome. Vanadium in different investigations showed anti-diabetic activity but until now an NZO mice model has not been tested with this element. The aim of this study was to investigate anti-diabetic activity of three vanadium compounds ($VOSO_4$, $VO(mal)_2$ and $Na(VO(O_2)_2bpy)\times 8H_2O$) in the NZO model. Metabolic syndrome was induced by special diet (1.5% of cholesterol and 15% of saturated fatty acids) during 8 weeks. In the next 5 weeks, the tested vanadium compounds were administered once daily, in a dose of 0.063 mmol/kg of body mass. At the end of the experiment, glucose, cholesterol, triglycerides and alanine transaminase were measured in the serum. The obtained results showed that the glucose level was decreased nearly to the healthy NZO mice in comparison to the NZO mice with metabolic syndrome. In all groups on the diet with cholesterol, the level of this parameter was statistically higher in comparison to the group without cholesterol addition. Vanadium treatment in a dose 0.063 mmol/kg of body mass does not influence cholesterol, triglycerides and alanine transaminase activity.

Keywords: New Zealand obese mice, vanadium, biochemical parameters

Metabolic syndrome (MS) is one of the most important problems in developing countries. This syndrome is associated with five principal conditions: large waistline, high triglyceride level, low HDL level, high blood pressure, high fasting blood sugar. Minimum three from the five risk factors mentioned above are the basis for recognizing this illness (1–3). MS increases risk of diabetes type 2 about five times and cardiovascular disease about two times in comparison with patients without MS (4). Diabetes, especially type 2, and cardiovascular diseases are among the main challenges for modern medicine and health care system not only due to the problem of treatment but also due to the number of cases, later multi-organs complication, as well as age and lifestyle of patients (5, 6). Prevention is one of the methods, which can significantly decrease the risk of MS and the associated health problems such as diabetes or cardiovascular diseases (7–10). Proper nutrition in both quality and quantity, an appropriate amount of physical activity are among the most important factors, which determine the likelihood of this syndrome. For the testing of new therapies or new medicines an appropriate animal model is necessary. One of them is New Zealand obese (NZO) mice. These mice can be used as a polygemic model of obesity, insulin resistance and hyperinsulinemia (11, 12). Especially fatty diet has an influence on the development of diabetes in these mice (13). Vanadium in different compounds has been tested as a potential anti-diabetic agent for about 30 years (14–17). In these investigations, organic compounds showed more interesting anti-diabetic activity than inorganic compounds (18, 19). Similar observation was reported on toxicity of this metal (20, 21). Some of organic complexes were

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tested also in a human study (15, 16) with positive results. Nowadays, some groups of researchers have synthesized novel organic compounds of vanadium, which can have less toxic effect with higher antidiabetic activity. However, for a better quality of investigation, it is necessary to have a good animal model to study the influence of vanadium on maintaining a proper glucose level. Diabetes type 2 is evidently associated with MS. Proper prevention is very important in diabetes development and it can postpone the moment to start a medicine treatment. Vanadium organic ligands can be useful potential agent in this period and also with other drugs treatment during diabetes.

MATERIALS AND METHODS

Vanadium compounds

1. $VOSO_4 \times H_2O$ – this compound of vanadium was purchased from Sigma.

2. $VO(mal)_2$ was prepared in the Faculty of Chemistry of the Jagiellonian University. The complex of $VO(mal)_2$ was prepared during synthesis under argon. To 5 mL of a hot aqueous solution containing 12 mmol of $VOSO_4 \times 5H_2O$ was added dropwise a hot solution of maltol 25 mmol in 25 mL of water. The pH of reaction mixture was adjusted to ca. 8.5 by addition of 2 M NaOH. The resulting mixture was refluxed with stirring for about 4 h and after cooling to room temperature the resulting green precipitate was filtered off, washed with cold water, and dried *in vacuo* at the room temperature.

3. $Na(VO(O_2)_2bpy) \times 8H_2O$ was also prepared in the Faculty of Chemistry of the Jagiellonian University, using 10 mmol of $NaVO_3$, which was dissolved in molar excess of 10% H_2O_2. The mixture contained molar ratio of H_2O_2 to vanadium 1 : 3. To the obtained solution cooled in the ice bath, 20 mL of ethanol solution containing 10 mmol of 2,2'-bipyridine was added. Temperature of the reaction mixture did not exceed 10°C during the synthesis. Afterwards, 50 mL of cooled ethanol was added. The solid phase was filtered and washed with 10 mL of cold ethanol. The synthesized vanadium complex was dried in the air, in a dark place.

Purity of these two compounds was confirmed by elemental analysis and infrared spectroscopy.

Animals

Fifty male NZO mice and twenty white CD1 mice, five weeks old, body mass 28–33 g were divided into seven groups of 10 animals (five per cage) as follows: – control NZO with standard diet (CN), control white CD1 mice strain with standard diet (CW), control NZO with fatty diet (FN), control white CD1 with fatty diet (FW) and three groups of NZO mice with tested vanadium compounds and fatty diet (V1FN, V2FN, V3FN). MS was induced during eight weeks by special diet containing 15% of saturated fats (lard) and 1.5% of cholesterol. The control group had standard diet. By the next five weeks, the investigated vanadium compounds were administered once daily by gavage in a dose of 0.063 mmol/kg of body mass in the volume of 10 mL/kg of body mass. During the vanadium administration, a composition of diet for all animals was not changed. Mice had all the time free access to water and feed. Day/night cycle was 12 h (7 a.m. – 19 p.m), temperature 22°C and humidity about 55 ± 10%. After thirteen weeks of the experiment, the animals were anesthetized (thiopental 60 mg/kg) and blood from abdominal aorta was taken, and after centrifugation (2500 rpm at 4°C), the serum obtained for biochemical parameters was collected and frozen at –80°C until analysis.

Biochemical analysis

All biochemical parameters were measured in serum using the standard Alize apparatus and standard kits for glucose, cholesterol, triglycerides, ALT, AST, ALP and uric acid (from Biomérieux), and it was controlled using Normal Control Serum – Seronorm and Pathological Control Serum – Pathonorm. The apparatus parameters used in these analyses were recommended by the manufacturer.

Statistical analysis

Statistical analysis of the obtained results was performed using Statistica 9 and GraphPad Prism software.

RESULTS

Glucose

Addition of saturated fats and cholesterol to diet of the investigated animals evidently increases statistically significantly glucose level (Fig. 1) in serum especially in NZO mice ($p < 0.001$, group FN). Also in white mice CD1 (FW group) an increase of this parameter was observed but was not significant ($p = 0.09$). Addition of all tested vanadium compounds decreases glucose level nearly to healthy NZO mice in comparison to NZO mice with fatty diet. This evident, visual decrease of glucose level in serum of tested animals with vanadium compounds treatment was not statistically significant because in different groups the scatter of indi-

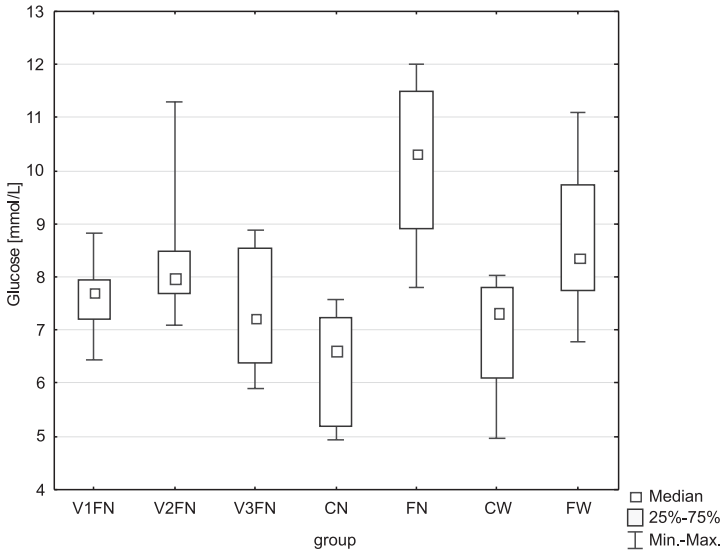

Figure 1. Glucose level in different groups of mice (V1FN, V2FN, V3FN – three NZO mice groups with tested vanadium compounds and fatty diet; CN – control NZO with standard diet; FN – control NZO with fatty diet; CW – control white CD1 mice strain with standard diet; FW – control white CD1 with fatty diet)

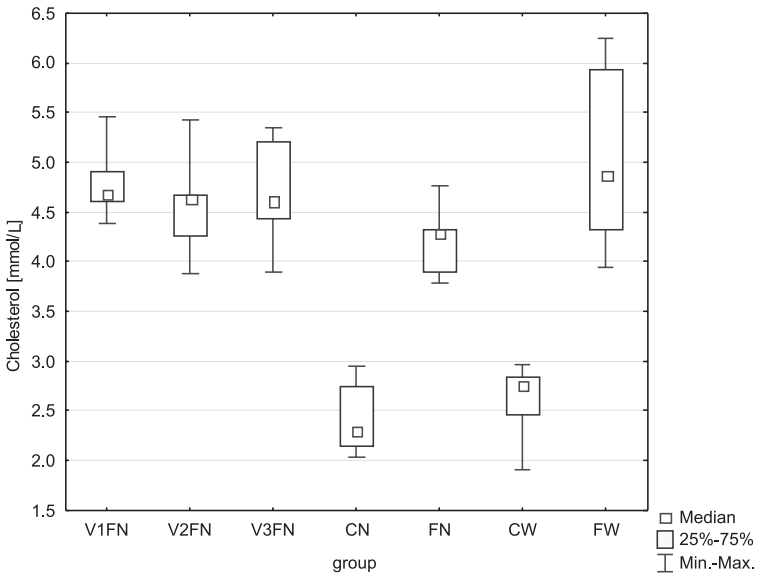

Figure 2. Cholesterol level in different groups of mice (V1FN, V2FN, V3FN – three NZO mice groups with tested vanadium compounds and fatty diet; CN – control NZO with standard diet; FN – control NZO with fatty diet; CW – control white CD1 mice strain with standard diet; FW – control white CD1 with fatty diet)

vidual results was large. However, this trend is interesting as the basis for future investigations of antidiabetic activity of vanadium treatment in the MS model.

Cholesterol

The diet rich in saturated fatty acids and cholesterol statistically increases the cholesterol level (Fig. 2) in all animal groups with this addition, in

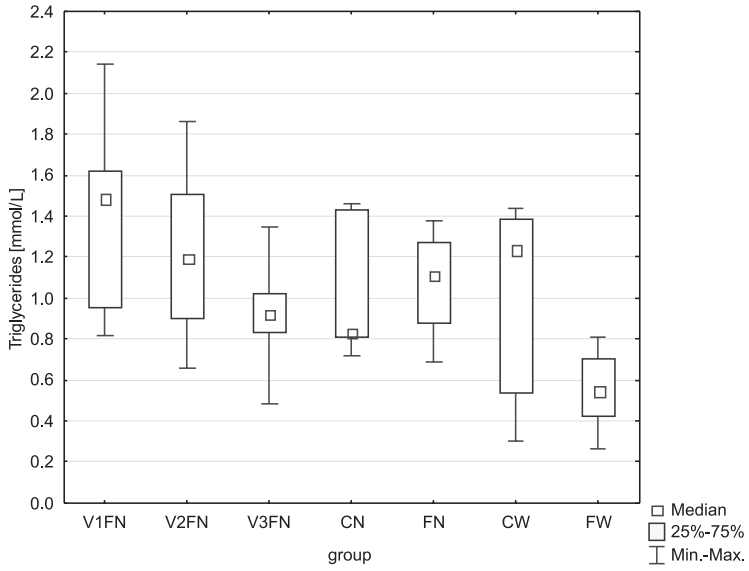

Figure 3. Triglycerides level in different groups of mice (V1FN, V2FN, V3FN – three NZO mice groups with tested vanadium compounds and fatty diet; CN – control NZO with standard diet; FN – control NZO with fatty diet; CW – control white CD1 mice strain with standard diet; FW – control white CD1 with fatty diet)

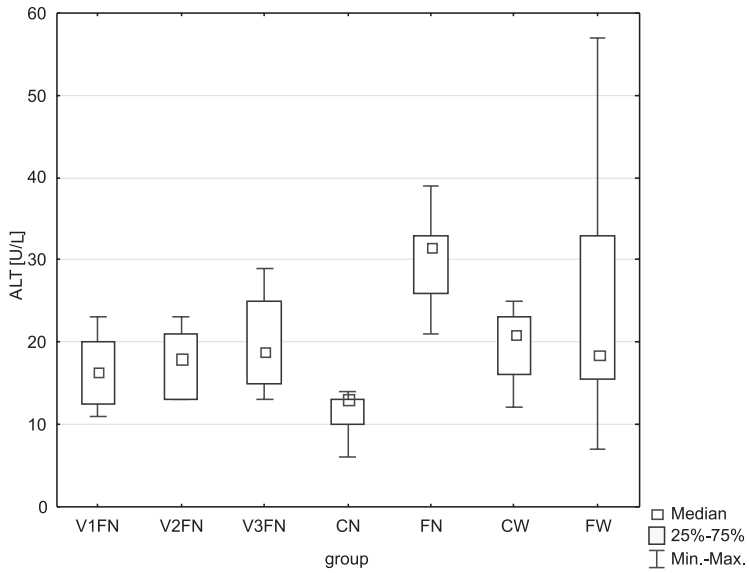

Figure 4. Alanine transaminase activity in different groups of mice (V1FN, V2FN, V3FN – three NZO mice groups with tested vanadium compounds and fatty diet; CN – control NZO with standard diet; FN – control NZO with fatty diet; CW – control white CD1 mice strain with standard diet; FW – control white CD1 with fatty diet)

comparison to the animals with standard feed ($p < 0.05$). The cholesterol level was about two times higher in the animals with modified diet (about 4.5 mmol/L *vs*. about 2.5 mmol/L) in comparison to the animals with standard diet (CN and CW groups). Between the CW and CN mice groups with standard feed, differences were not observed. Vanadium treatment in NZO mice with fatty diet (groups V1FN, V2FN, V3FN) showed not statistical increase (about 20%) of the cholesterol level in comparison to NZO mice with fatty diet (FN group).

Triglycerides

The triglycerides level was similar in all groups of animals (Fig. 3). Only one statistically significant difference was observed between CD1 mice with fatty diet in the FW group and all NZO mice with fatty diet and vanadium treatment in the V1FN, V2FN, V3FN groups. An influence of saturated fatty acids on triglycerides level decrease in white CD1 strain mice (FW group) was also observed. This can suggest a different response associated with the strain of mice used in the experiment.

Alanine transaminase (ALT)

The activity of ALT (Fig. 4) was statistically higher in the NZO mice (FN) with fatty diet in comparison to the control NZO mice (CN). An addition of vanadium compounds to the NZO mice with fatty diet decreased the activity of this enzyme nearly to the level of the CN group. It was not significant but the trend was spectacular. Between the CW and FW groups there were no differences in the ALT activity but a difference between the strains of mice was observed. The ALT activity in the CW mice was higher in comparison to the CN group.

Aspartate transaminase (AST), alkaline phosphatase (ALP), and uric acid

As for the other investigated parameters, namely aspartate transaminase (AST), alkaline phosphatase (ALP), and uric acid, differences between groups were not observed (Tab. 1). The observed very large variability between animals in investigated groups preclude any valid interpretation of the data.

DISCUSSION

Diabetes type 2 is associated with insulin resistance and relative insulin deficiency. Usually, it is associated with the age of patients and other diseases. Proper prevention, such as healthy diet and physical exercise, can frequently significantly delay the start of drug therapy (22). For prevention, vanadium compounds, which showed antidiabetic activity in different scientific researches (15–21, 23–25) can be used. In animal models, vanadium was usually used in fully developed diabetes, both types 1 and 2. Using this microelement in the early stages of the diabetes development is interesting from the scientific and therapeutic point of view. MS in an animal model can be a suitable point for this study. In the New Zealand obese (NZO) mice with special fatty diet insulin resistance, hyperglycemia and abdominal obesity increase more quickly than in the NZO mice with standard diet (26). This relationship is very useful for the study of an initial stage of diabetes type 2. Incorrect diet rich in cholesterol and saturated fatty acids is frequently one of key factors of MS and subsequently diabetes type 2. Till now, the NZO mice's MS and vanadium compounds were not examined together. The obtained results of the glucose level confirmed statistical influence of fatty diet on the level of this parameter and they are consistent with the work of other authors (27–29). In both CD1 and NZO mice after fatty diet, the glucose level was higher in comparison to these mice with standard diet. An increase was more spectacular in the case of the NZO mice. Unfortunately, to the best of our knowledge for this moment, the comparison

Table 1. Activity of aspartate transaminase (AST), alkaline phosphatase (ALP) and uric acid level in serum in different groups of animals.

Group	AST [U/L]	ALP [U/L]	Uric acid [mmol/L]
V1FN	80 ± 15	26 ± 4	1.13 ± 0.50
V2FN	72 ± 13	27 ± 5	1.03 ± 0.48
V3FN	76 ± 10	28 ± 5	1.22 ± 0.58
CN	83 ± 14	23 ± 5	1.14 ± 0.45
FN	86 ± 20	24 ± 4	1.55 ± 0.74
CW	87 ± 20	26 ± 5	1.06 ± 0.33
FW	85 ± 16	29 ± 6	1.20 ± 0.41

V1FN, V2FN, V3FN – three NZO mice groups with tested vanadium compounds and fatty diet; CN – control NZO with standard diet; FN – control NZO with fatty diet, CW – control white CD1 mice strain with standard diet, FW – control white CD1 with fatty diet.

of influence of high fatty diet on the glucose level in both mice strain is not presented in the Pubmed base. A higher level of glucose in the NZO mice with high fat diet confirms the findings of other researchers concerning the usefulness of this model in the MS and diabetes study. All vanadium compounds (V1, V2 and V3) used in the present investigation showed an anti-diabetic – but not significant – effect. The results obtained for the glucose level after the vanadium treatment showed that a very small administered dose of different compounds (0.063 mmol/kg of body mass or 3.21 mg V/kg of body mass had anti-diabetic activity and was one of smaller doses used in vanadium anti-diabetic investigations. The used dose was chosen as 1/20 LD_{50} for $Na(VO(O_2)_2bpy) \times 8\ H_2O$ obtained in other investigation with streptozotocin-diabetic rats (30). For all three vanadium compounds, the same dose was used to observe potential differences of these compounds. Higher doses of the selected vanadium compounds can probably give more interesting statistical differences. It should be noted that the present work is the first study of this animal model with vanadium compounds and, therefore, it cannot be compared to other similar studies. In the work of Xie at al. (31) an administered dose was 10 and 20 mg V/kg, in the work of Yanadarg et al., an administered dose was 0.2 mmol/kg (32), in the work of Gao et al. an administered dose was 0.1, 0.2 and 0.4 mmol/kg (33). Using a small dose of vanadium can eliminate the toxic effect of this metal reported sometimes by other authors (20, 21). This suggests that the NZO mice model of MS with vanadium treatment can be useful in pre-diabetes and early stage of diabetes investigations. The reaction of the glucose level after vanadium treatment in the NZO mice model is interesting for the future studies of new vanadium compounds and other anti-diabetic substances. The choice of a suitable dose of investigated compounds is a very important issue, which can be investigated in the future studies.

The second observation is an important influence of diet on the cholesterol level. High fat diet with 1.5% of cholesterol significantly increases this parameter in the group of animals with fat and cholesterol addition. The results are similar to the work of Irwin et al. and Zhou et al. (34, 35). These authors report that high fat diet increases statistically the total cholesterol level. In our work, diet which contained additionally 1.5% of cholesterol increased of total cholesterol in mice blood higher (about 4.0–4.7 mmol/L) than in the case of solely high fat diet in the work of Irwin and Zhou (3.5–4.1 mmol/L). In the CD1 mice, an increase of total cholesterol was higher but not significant in comparison to the NZO mice after high fat diet. This result can speak about genetic differences between both mice strains for high fat diet treatment. Also in this case, to the best of our knowledge for this moment, a comparison of influence of high fat diet on the cholesterol level in both mice strains is not presented in the Pubmed base. Treatment of vanadium in NZO mice with high fat diet minimally increases the cholesterol level – about 0.5 mmol/L – in the blood in comparison to vanadium not treated mice with high fat diet. Some works reported a lowering effect of vanadium for the cholesterol level (36–39). This small increase of the cholesterol level in blood in our case can be associated with a different model of animals or with changes of proportion of the HDL and LDL level. If it is lowering of LDL fraction, it is interesting action of vanadium in this model. If it is associated with lowering HDL fraction, this organism response to vanadium treatment can be dangerous. The work of Ramachandran et al. (40) presents an increase of the HDL level in STZ rats after vanadium treatment. Probably in our case there is also the same mechanism but a response to this question can be given by further investigation, extended to a full lipid profile as well as all cholesterol fractions and triglycerides. Triglycerides are one of the parameters, which has an important role in cardiovascular disease and diabetes development. High fat diet used in the present experiment does not influence this parameter and the obtained results are similar to the work of other authors (41–44).

The activity of alanine transaminase in animal blood after vanadium administration was lower in comparison to vanadium not treated animals. This can suggest that the doses of vanadium, which were used did not have toxic effects and do not influence the liver function. The activity of ALT was investigated by Pepato et al. (45). These authors observed an increase of the activity of ALT but vanadium doses used in the investigation were about 10 times higher that in our experiment.

As for the other investigated parameters, namely aspartate transaminase (AST), alkaline phosphatase (ALP) and uric acid, differences between groups were not observed. This can suggest that the used dose of vanadium compounds does not have negative effects on the examined parameters and can be used in higher doses in similar future investigations.

The obtained results demonstrated the potential of the NZO mice model in anti-diabetic investigations of vanadium compounds in the starting stage of MS and, consequently, diabetes. The present

work was a preliminary study to find a possible preventive action by vanadium compounds in the development of MS and diabetes. For a better understanding of the interaction of the NZO mice model and vanadium treatment, it is necessary to make also other investigations in blood, especially such as glucose – insulin tolerance test, plasma insulin level, different doses of vanadium compounds, insulin sensitivity. Other interesting areas are to investigate blood pressure, atherosclerotic lesions in aorta and oxidative changes in organs. Potential use of vanadium compounds in MS treatment may delay the time of the application of drugs, which stimulate insulin secretion. It is very important for elderly patients because insulin application in the form of injections is burdensome for them.

CONCLUSION

The obtained results suggest an interesting biological activity of vanadium compounds in very small doses in the MS model. This shows a possibility of using vanadium compounds as an anti-diabetic agent, especially in an early stage of diabetes development.

Also the NZO mice model can be useful in the diabetes development study.

Acknowledgments

This work was supported by Grant No. K/ZBW/000497 of Medical College of Jagiellonian University. We would also want to give special thanks to technicians: Ewelina Gajdzik, Barbara Tatar and Iwona Zagrodnik for their help during this project realization.

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Received: 25. 10. 2013