ACTA BIOLOGICA CRACOVIENSIA Series Zoologia 54: 19–25, 2012

The Influence of Vanadium (V) on the Ultrastructure of Hepatocytes of the Rat Liver

Anna Kopacz-Bednarska*, Teodora Król*[1], Halina Zaporowska**, Ewa Trybus* and Wojciech Trybus*

** Department of Cell Biology, Institute of Biology, The Jan Kochanowski University, Świętokrzyska 15, 25-406 Kielce, Poland*
*** Department of Cell Biology, Institute of Environmental Protection, The John Paul II Catholic University in Lublin, Kraśnicka 102, 20-718 Lublin, Poland*

Accepted October 31, 2012

Vanadium is an element which shows a multidirectional mechanism of action, it has a positive influence on the physiological and biochemical processes of the body, however at high concentrations it can show toxic properties. For that reason it is important to assess the influence of a specific dose and action time of vanadium on the morphological profile of rat hepatocytes. The purpose of this research was to evaluate the changes in β-D-glucuronidase activity and the ultrastructural profile of rat hepatocytes subject to 18 weeks of sodium metavanadate in the concentration of 0,125 mg V ml^{-1}. The experimental animals were 2-month-old male Wistar rats. The biochemical analysis was done based on the distribution of synthetic substrates using a spectrophotometric method. The biochemical research results revealed a statistically significant reduction in the activity of β-D-glucuronidase (β-Gr) in the liver cells of experimental animals. The submicroscopic assessment of the cells was performed with the use of a transmission electron microscope. In the ultrastructural image of the hepatocytes of experimental animals, changes associated mainly with mitochondria, the lysosomal system and peroxisomes were observed.

Keywords: sodium metavanadate, β-D-glucuronidase, lysosome, hepatocyte, ultrastructuren

INTRODUCTION

Recently, there has been a growing interest in the influence of various chemical elements on the physiological processes of the organism. Numerous research publications concentrate on determining the range of activity of bioelements in metabolic reactions. One of such elements is vanadium – commonly appearing in plant and animal tissues.

Vanadium is an element from the 5th group of the periodic table; its atomic number is 23 and molar mass 50.942. The existence of vanadium was first suggested in 1801 by a Mexican

[1] tkrol@op.pl

PL ISSN 0001-530X

chemist named A.M. del Rio, who was unable to scientifically prove his discovery, which was only done in 1830 by Nils Sefstrom, a Swedish scientist (Mukherjee et al., 2004; Kordowiak and Holko, 2009). Vanadium is a microelement displaying a multidirectional mechanism of action. It influences, for example, the metabolism of cholesterol, lipids (including phospholipids), carbohydrates and the activity of numerous enzymes (Gromysz-Kałkowska and Szubartowska, 1999). There are also some promising results of experimental research confirming the insulin-like properties of vanadium compounds (Sakurai et al., 1995; Cadène et al., 1996; Dahmane et al., 1997; Gupta et al., 1999; Crans, 2000; Sakurai et al., 2004). It has been proven that sodium orthovanadate, when administered in drinking water, at a concentration of 0.6 mg ml^{-1} to rats with alloxan-induced diabetes, effectively regulates the activity of key enzymes associated with the metabolism of the body's sugar-lipid balance, which stabilizes the blood glucose levels to a point similar to the animals receiving insulin (Gupta et al., 1999). It has been shown that vanadium compounds, like insulin, normalize the blood glucose level mainly by the intensified transport of glucose in tissues through the effect of vanadium compounds on glucose transporters GLUT-4 with simultaneous inhibition of the processes of glycogenolysis and gluconeogenesis (Kiersztan, 1998; Kordowiak and Holko, 2009).

Vanadium compounds, besides having a positive influence on the physiological and biochemical processes of the body, can show toxic properties at high concentrations. For that reason it is important to assess the influence of a specific dose and action time of selected vanadium compounds on the morphological profile of rat hepatocytes.

MATERIALS AND METHODS

The study was performed on 2-month-old male Wistar albino rats. The animals were kept under standard laboratory conditions in an air-conditioned room with a naturally regulated light to dark ratio (LD 12:12), at a constant temperature (20-21^{0}C) and relative air humidity (55 ± 5 %). The animals were divided into two groups: group I (control), which received deionized water to drink each day and group II (experimental) receiving $NaVO_3$ (SMV; Sigma, St. Louis, MO, USA) aqueous solution at a concentration of 0.125 mg V ml^{-1}. The animals from group II received the investigated agent for 18 weeks, while the control animals were analogously given deionized water, prepared with the use of Aries deionizer (Resin Tech., Inc., USA).

The animals were given standard granulated rodent feed (Labofeed B; Fodder and Concentrate Factory, Kcynia, Poland). For the whole experimental period, the feed and deionized water were available ad libitum. During the experiment, the provision of food, fluids and water, as well as the general conditions of both the control and the experimental group were monitored every day. After the experiment had been completed, liver segments were taken for morphological and biochemical studies. The liver segments used for electron microscopy were fixed in 3% glutaraldehyde, contrasted with 2% OsO_4, and then immersed in epoxy resin Epon 812 (Serva, Germany). The fixation, contrasting and immersion of liver segments for electron microscopy were done according to the method described by Marzella and Glaumann (1980a). Ultra thin slices were prepared with the use of glass knives in the Leica EM UC7 ultramicrotome. Photographs were made with the use of the Tecnai G2 Spirit transmission electron microscope (FEI Company).

Liver tissue homogenates were obtained according to the method of Marzella and Glaumann (1980b). Suitably minced liver tissue was suspended in 0.25 M saccharose. The whole sample was then homogenized in a Teflon piston homogenizer. The liver homogenates were centrifuged in the Sorvall RC 6 Plus centrifuge (Termo Scientific, Waltham, MA, USA) for 10 minutes at 700g. In the obtained liver tissue supernatant, the activity of β-D- glucuronidase (β-Gr, EC 3.2.1.31) was assayed using the method of Barrett (1972) and the total protein content by the method of Lowry, modified by Kirschke and Wiederanders (1984). The biochemical studies were performed with the use of Sigma reagents (St. Louis, MO, USA). The extinction of the investigated enzymes was read with the use of the UV-VIS Spekol 1500 spectrophotometer (Analytik Jena). The activity of the investigated enzymes was expressed in µmoles for mg of $protein^{-1}$ h^{-1} and presented in the form of averages and appropriate mean deviations. The statistical

analysis was done using the STATISTICA 6.0 software. The chosen level of statistical significance was $p<0.05$.

This research was accepted by the 1st Local Animal Ethics Committe in Lublin.

RESULTS

This study analyzed the influence of a water solution of $NaVO_3$ (SMV) administered into the drinking water of the studied animals for 18 weeks in the concentration of 0,125 mg V ml^{-1} on the ultrastructural profile and the activity of β-D-glucuronidase in the liver cells of studied animals. The obtained biochemical results are shown in Table 1, while the submicroscopic changes observed through TEM are shown on Figs. 1, 2 and 3.

The ultrastructure of hepatocytes taken from control group rats was used for the normal image of the liver cell (Fig.1). The analysis of the morphological profile of animal hepatocytes under a long-term activity of SMV (Figs. 2, 3) revealed significant changes in comparison to the ultrastructure of control group rats (Fig.1) Figs. 2 and 3 show a normal structure of the cellular nucleus, without any indications of the expansion of the nuclear membrane, with a normal structure of the nucleolus. Furthermore, in comparison to the submicroscopic profile of the control cells (Fig.1), encumbering the animals with sodium metavanadate for 18 weeks caused an increase in the number of peroxisomes

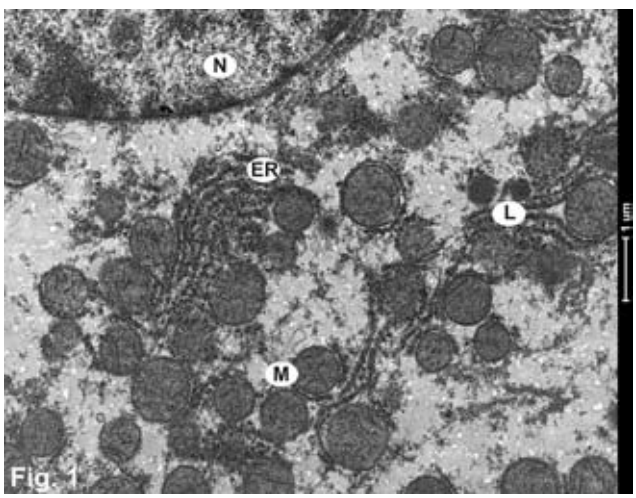

Fig. 1. Hepatocyte fragment from a control group rat. (ER) endoplasmic reticulum, (M) mitochondria, (N) nucleus, (L) lysosome. × 11 500

TABLE 1. The influence of 18 weeks of $NaVO_3$ (SMV) action on the activity of β-D-glucuronidase (β-Gr) in rat liver.

Enzyme	Control		[%]	$NaVO_3$ (SMV)		[%]
	average	SE		average	SE	
β-Gr	0,74	0,12	100%	0,37	0,10	**50%** [a]

Statistical differences confirmed at: [a] $p<0,001$ (ANOVA/Tukey's test)

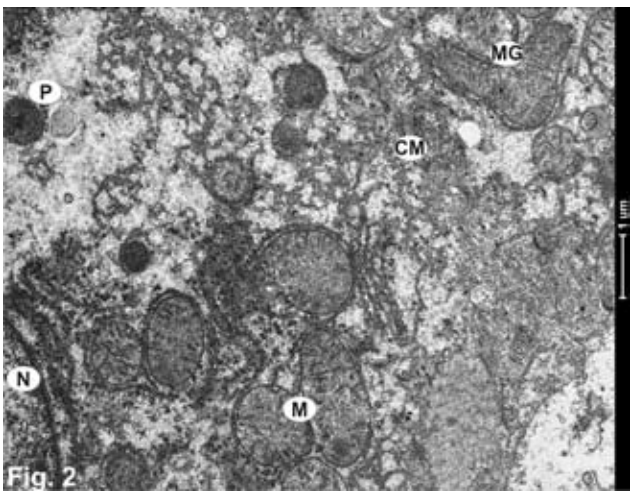

Fig. 2. Hepatocyte fragment from a rat receiving $NaVO_3$ in the concentration of 0,125 mg V ml^{-1} for 18 weeks. (CM) cellular membrane, (M) mitochondria, (MG) megamitochondria, (N) nucleus, (P) peroxisome. × 11 500

(Fig. 3). The large number of mitochondria present should also be noted. Changes in their structure show mainly through the increase in their number and size with the simultaneous maintenance of the normal crest arrangement and normal density of the mitochondrial matrix. Furthermore, Fig. 2 show structures known as megamitochondria, which have probably been created through the fusion of adjacent organelles. In the submicroscopic image of the studied hepatocytes, no elements of the smooth endoplasmic reticulum and a reduced compartment of the rough endoplasmic reticulum were observed in response to SMV activity (Fig. 2,3), which reflects the results obtained through biochemical analysis.. The results of biochemical studies revealed a reduction in the activity of the studied lysosomal enzyme in rat liver, caused by the activity of a water solution of SMV administered in the concentration of 0,125 mg V ml^{-1} (Tab.1).

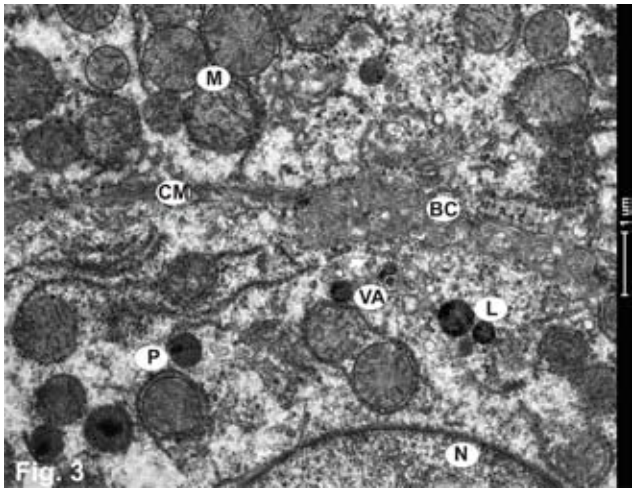

Fig. 3. Hepatocyte fragment from a rat receiving $NaVO_3$ in the concentration of 0,125 mg V ml^{-1} for 18 weeks. (BC) bile canaliculus, (CM) cellular membrane, (M) mitochondria, (N) nucleus, (L) lysosome, (P) peroxisome, (VA) autophagic vacuole. × 11 500

DISCUSSION

Current research literature gives a lot of attention to issues concerning the influence of numerous elements on cell metabolism. Bioelements, when at appropriate concentrations, play a very important role in humans and animals. Vanadium is one of such elements. At micromolar concentrations it is necessary for proper growth and development of the body (Chakraborty et al., 2007). Trzos and Kordowiak (1994) state that vanadium concentration in the body varies depending on the tissue, and it ranges from 0.1 to 1μM. The daily dietary requirement for vanadium is about 10-20μg (Domingo et al., 1996; Aleksandrowa et al., 1999), and its consumption is estimated to be around 10-60μg (Bichard and Henquin, 1995; Domingo, 1996). The fact that vanadium concentration in food is around 1 ng g^{-1} indicates that the risk of disorders arising from the deficiency of this element in the human body is insignificant (Evangelou, 2002). However, due to the wide use of vanadium derivatives in different industries and the associated possibility of an increase in its concentration in the tissues, there is an increasing possibility of a potentially toxic action of vanadium on the body.

Kordowiak and Holko (2009) report that the diverse mechanism of action of vanadium derivatives and their toxicity are largely dependent on the examined cells, the valence and concentration, and method of administration of vanadium, as well as on the duration of the experiment. According to the literature, pentavalent vanadium compounds display the highest toxicity towards cells (Kordowiak et al., 2007; Holko and Kordowiak, 2009). This is the reason for using the inorganic vanadium compound – sodium metavanadate ($NaVO_3$) – in this experiment. The human body contains around 100 μg vanadium; the majority of the element accumulates in bones, lungs, kidneys, spleen and liver (Aleksandrowa, 1999; Evangelou, 2002). According to the current literature, the greatest amounts of vanadium compounds accumulate in the cellular nucleus and mitochondria (Zaporowska, 1995). For this reason the potentially toxic action of vanadium should be expected to change these cellular structures. The results of our research (Fig. 2 and 3) show a slightly changed cellular nucleus (there were no significant changes in the nuclear membrane or the nucleolus), which might suggest a very low level of cellular accumulation of the investigated agent used at that dose. However, this research has not shown (Fig. 2 and 3) that administration of $NaVO_3$ at a dose of 0.125 mg V ml^{-1} causes distinct changes in the structure of mitochondria in comparison with the ultrastructural profile of control cells (Fig. 1). It has been demonstrated that after 18 weeks of $NaVO_3$ activity there is a significant increase in the number and size of mitochondria in the hepatocytes of the investigated rats, while at the same time the correct cristae arrangement and mitochondrial matrix density are preserved (Fig. 2 and 3), which is most probably the response of rat liver cells to the increase in energy requirements resulting from the encumbrance of the cell with the investigated compound. Furthermore, Fig. 2 shows the existence of distinctly swollen mitochondria, which in consequence might lead to the formation of structures known as megamitochondria which are probably formed as a result of peroxidative $NaVO_3$ activity. As indicated by Karbowski et al. (1999), Teranishi et al. (2000) and Wakabayashi (2002), the formation of megamitochondria might be the result of the cellular response to unfavorable conditions, in this case to the increased oxygen free radicals (OFR). As shown by the above-mentioned authors, the prolonged activity of free radicals might lead to a decrease in the mitochondrial transmembrane potential ($\Delta\Psi m$), which started changes leading to

the induction of apoptosis. In our research, the increased number of peroxisomes visible in the submicroscopic image of hepatocytes encumbered with $NaVO_3$ (Fig. 2 and 3), as compared with the ultrastructure of the cells from the control group (Fig. 1), could be the expression of oxygen free radicals being generated by the vanadium compound. The changes observed in the ultrastructural image are confirmed by available literature and could indicate a diverse mechanism of action of vanadium derivatives. On the one hand, OFRs might cause oxidative damage to cellular microparticles, which could potentially even lead to cancerous changes (Ścibior-Bentkowska and Czeczot, 2009). On the other hand, the oxidative stress could, in part, reveal potential antineoplastic properties of vanadium. The available literature shows that compounds which directly or indirectly generate reactive forms of oxygen (RFO) through the induction of numerous cellular injuries which, in effect, lead to cell death, might be used in the therapy of oncological diseases (Ścibior-Bentkowska and Czeczot, 2009). It is believed that the exposure of neoplastic cells to prolonged action of drugs generating RFO might lead to the exhaustion of their defensive antioxidative materials and, as a result, lead to cell death (Ścibior-Bentkowska and Czeczot, 2009).

In order to study thoroughly the changes occurring at the cellular level under the influence of the investigated vanadium compound, the submicroscopic studies were supplemented with the analysis of the level of enzyme activity. Rat liver cells exposed for 18 weeks to the activity of $NaVO_3$ at a concentration of 0.125 mg V ml^{-1} showed significant changes in the activity of the model enzyme of the lysosomal compartment, β-D-glucuronidase (Table 1). This enzyme is a glycosidase catalyst for the hydrolysis of natural or synthetic β-D-glucuronides which are formed through the fusion of β-D-glucuronic acid and appropriate aglycones (Hoja-Łukowicz, 1999), and according to Morita et al. (2008) the hydrolysis of glucuronic acid conjugates can also lead to the release and activation of compounds with potential carcinogenic properties. The analysis of the enzymatic activity in cells exposed to $NaVO_3$ action shows a statistically significant inhibition of the activity of β-Gr (lower by 0.37 μmol at $p = 0.000890$) in comparison with control values, indicating a decreased ratio of β-D-glucuronide decomposition in the investigated hepatocytes. The obtained biochemical results were confirmed by the ultrastructural image of the liver cells from experimental animals (Fig. 2 and 3) in which there was no increase in the amount of smooth endoplasmic reticulum (SER), the main area associated with the detoxification of malignant metabolites (Madej, 2003). It is known that SER also participates in the process of glycogeneolysis. The reduction of the SER compartment is mainly associated with the inhibition of the activity of glucose-6-phosphatase, and through it, the process of glycogen decomposition. These data are confirmed by the research carried out by the team of GUPTA (Gupta et al., 1999), where sodium orthovanadate, used at a concentration of 0.6 mg ml^{-1} led to the inhibition of glucose-6-phosphatase activity, which might also explain the insulin-like mechanism of vanadium action.

At present there are no sufficient data on the influence of vanadium compounds on the morphological profile of normal cells. The current ultrastructural research, performed with the use of transmission electron microscopy and concerning other compounds, shows that such studies provide extremely valuable information regarding the influence of different chemical substances or pharmaceutical compounds on the submicroscopic image and the function of specific cellular structures.

The experimental publications published in recent years indicate that vanadium and its derivatives show a wide spectrum of biological activity. However, the mechanism of vanadium action on the cellular level still remains insufficiently explained; therefore its potential pharmacological and therapeutic properties require further study.

CONCLUSIONS

1. Sodium metavanadate administered to animals in drinking water at a concentration of 0.125 mg V ml^{-1} for a period of 18 weeks caused an increase in the number of peroxisomes and the formation of megamitochondria, which probably resulted from the free-radical influence of the investigated compound.

2. The lack of smooth endoplasmic reticulum and distinct changes in the cellular nucleus are, however, signs of low hepatotoxicity of the administered $NaVO_3$ dose.

3. The activity of $NaVO_3$ affected a reduction in β-Gr activity, indicating an inhibition of the glucuronidation process in the rat liver cells.

REFERENCES

Alexandrova, R. 1999. The secrets of vanadium. *Exp. Pathol. Parasitol. 2: 39-44.*

Barrett, A.J. 1972. Lysosomal enzymes. In Lysosomes. A laboratory Handbook (J.T.Dingle Ed.) *North-Holland Pub. Com., Amsterdam, 46-135.*

Brichard, S.M, and J.C. Henquin. 1995. The role of vanadium in the management of diabetes. *Trends Pharmacol. Sci. 16 (8): 265-270.*

Cadène, A., R. Gross, P. Paucheret, J.J. Mongold, P. Masiello, M. Roye, G. Ribes, J.J. Serrano, and G. Cros. 1996. Vanadyl sulphate differently influences insulin response to glucose in isolated pancreas of normal rats after in vivo or in vitro exposure. *Eur. J. Pharmacol. 318 (1): 145-151.*

Chakraborty, T., A. Chatterjee, A. Rana, D. Dhachinamoorthi, P.A. Kumar, and M. Chatterjee. 2007. Carcinogen-induced early molecular events and its implication in the initiation of chemical hepatocarcinogenesis in rats: Chemopreventive role of vanadium on this process. *Biochim. Biophys. Acta 1772: 48–59.*

Crans, D.C. 2000. Chemistry and insulin-like properties of vanadium (IV) and vanadium (V) compounds. *J. Inorg. Biochem. 80: 123-131.*

Dahmane, A., S. Gil, F. Croquet, J. Davy, and J. Féger. 1997. Differential effect of vanadate on receptor-mediated endocytosis of the asialoglycoprotein receptor in hepatocytes from normal and diabetic rats. *Biochem. Pharmacol. 54: 349-355.*

Domingo, J.L. 1996. Vanadium: a review of the reproductive and developmental toxicity. *Reprod. Toxicol. 10 (3): 175-182.*

Evangelou, A.M. 2002. Vanadium in cancer treatment. *Crit. Rev. Oncol. Hemat. 42: 249–265.*

Gromysz-Kałkowska, K., and E. Szubartowska. 1999. Dwa oblicza wanadu. *Wszechświat 100 (9): 185-189.*

Gupta, D., J. Raju, R.J. Prakash, and N.Z. Bauer. 1999. Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats: effect of insulin and vanadate. *Diabetes Res. Clin. Pr. 46: 1–7.*

Hoja-Łukowicz, D. 1999. Transport kwaśnych hydrolaz do lizosomów na przykładzie β-glukuronidazy. *Kosmos 48 (1): 117-126.*

Holko, P., and A.M. Kordowiak. 2009. Pochodne wanadu jako związki o istotnym znaczeniu biologicznym. Część II. Wpływ na komórki nowotworowe. *Post. Biol. Kom. 36 (3): 377-394.*

Karbowski, M., C. Kurono, M. Wozniak, M. Ostrowski, M. Teranishi, Y. Nishizawa, J. Usukura, T. Soji, and T. Wakabayashi. 1999. Free radical-induced megamitochondria formation and apoptosis. *Free Radic. Biol. Med. 26(3-4): 396-409.*

Kiersztan, A. 1998. Insulinopodobne działanie związków wanadu. *Post. Biochem. 44 (4): 275-281.*

Kirschke, H., and B. Wiederanders. 1984. Methoden zur Aktivitätsbestimmung von Proteinases. *Martin-Luther Universität, Halle-Wittenberg, Wissenschaftliche Beiträge, Halle/ Salle 11-17.*

Kordowiak, A.M., A. Klein, A. Goc, and W. Dąbroś. 2007. Comparison of the effect of $VOSO_4$, Na_3VO_4 and $NaVO_3$ on proliferation,viability and morphology of H35-19 rat hepatoma cell line. *Pol. J. Pathol. 58 (1): 51–57.*

Kordowiak, A.M., and P. Holko. 2009. Pochodne wanadu jako związki o istotnym znaczeniu biologicznym. Część I. Działanie przeciwcukrzycowe. *Post. Biol. Kom. 36 (3): 361-376.*

Madej, J.A. 2003. Podstawy cytopatologii. *Wyd. Med. Urban & Partner, Wrocław: 91.*

Marzella, L., and H. Glaumann. 1980a. Increased degradation in rat liver induced by vinblastine II: Morphological characterization. *Lab. Invest. 42: 8-27.*

Marzella, L., and H. Glaumann. 1980b. Increased degradation in rat liver induced by vinblastine I: Biochemical characterization. *Lab. Invest. 42: 8-17.*

Morita, N., Z. Walaszek, T. Kinjo, T. NishiMaki, M. Hanausek, T.J. Slaga, H. Mori, and N. Yoshimi. 2008. Effects of synthetic and natural in vivo inhibitors of β-glucuronidase on azoxymethane-induced colon carcinogenesis in rats. *Mol. Med. Rep. 1:741-746.*

Mukherjee, B., B. Patra, S. Mahapatra, P. Banerjee, A. Tiwari, and M. Chatterjee. 2004. Vanadium—an element of atypical biological significance. *Toxicol. Lett. 150: 135–143.*

Sakurai, H., K. Fujii, H. Watanabe, and H. Tamura. 1995. Orally active and long-term acting insulin-mimetic vanadyl complex: bis(picolinato)oxovanadium (IV). *Biochem. Bioph. Res. Co. 214 (3): 1095-1101.*

Sakurai, H., T. Inohara, Y. Adachi, K. Kawabe, H. Yasui, and J. Takada. 2004. A new candidate for insulinomimetic vanadium complex: synergism of oxovanadium(IV)porphyrin and sodium ascorbate. *Bioorg. Med. Chem. Lett. 14: 1093–1096.*

Ścibior-Bentkowska, D., and H. Czeczot. 2009. Komórki nowotworowe a stres oksydacyjny. *Post. Hig. Med. Dośw. 63: 58-72.*

Teranishi, M., J.H. Spodonik, M. Teranishi, J.H. Spodonik, M. Karbowski, C. Kurono, T. Soji, and T. Wakabayashi. 2000. Swelling of free-radical-induced megamitochondria causes apoptosis. Exp. Mol. Pathol. *68 (2): 104-123.*

Trzos, R., and A.M. Kordowiak. 1994. Derivatives of vanadium as insulin-mimetic agent in diabetes. *Post. Biol. Kom. 21 (3): 293-302.*

Wakabayashi, T. 2002. Megamitochondria formation – physiology and pathology. *J. Cell. Mol. Med. 6 (4): 497-538.*

Zaporowska, H. 1995. Rola wanadu w metabolizmie komórki zwierzęcej. *Post. Biol. Kom. 22 (2): 149-165.*