Arh.farm 2010;60: 1 – 12 *Originalni naučni rad /Original scientific papes*

Low concentration of ammonium decavanadate exhibited insulin mimetic effect on isolated rat adipocytes

Aleksandra Topic[a*], Marina Milenkovic[b], Snežana Uskokovic-Markovic[c], Dragana Vucicevic[b]

[a] Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia
[b] Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, Serbia
[c] Department of Analytical Chemistry, Faculty of Pharmacy, University of Belgrade, Serbia

Summary

In this study, the insulin mimetic activity of ammonium decavanadate (DV) (in concentration 0.1 and 1.0 mM) dissolved in saline (0.9 % w/v of NaCl) or dimethyl sulfoxide, DMSO (2% v/v) was evaluated by means of *in vitro* measurements of the glucose uptake (GU) and inhibition of free fatty acids release (IFFAR) using isolated epinephrine-pretreated white adipocytes from Wistar rats.

Our data showed strong insulin mimetic effect of DV (> 80 %) in both concentration and independently of the solvent were used.

Having in mind satisfactory pharmacological effect of DMSO as well as its capability of penetration enhancer it would be reasonable to continue *in vivo* testing of dose-dependent insulin mimetic effect of decavanadate in DMSO. It is also necessary to examine toxicity of decavanadate in order to estimate its therapeutic index as potential antidiabetic agent.

Key words: decavanadate, adipocytes, insulin mimetic, dimethyl sulfoxide

* Corresponding author: **Aleksandra Topic,** Institute of Medical Biochemistry, Faculty of Pharmacy, Vojvode Stepe 450, 11 000 Belgrade, Serbia
Tel: +381 11 39 51 283, Fax: +381 11 39 72 840, E-mail: **atopic@pharmacy.bg.ac.rs**

Introduction

Diabetes mellitus (DM) is the most common endocrine disease. The disease is characterized by metabolic abnormalities and by long-term complications involving the eyes, kidneys, nerves, and blood vessels. It is associated with reduced life expectancy, significant morbidity due to specific diabetes related acute and chronic complications.

In recent years, it is a growing interest in examining of potential insulin mimetic metallopharmaceuticals based on characteristic properties of the metal ions.

Studies of biomedical activity of inorganic compounds like molybdenum, tungstate and vanadium have attracted the attention of numerous investigators [1-4].

It has been shown that in millimolar concentration range the oligoanions of V (V), W (VI) and Mo (VI) are potent inhibitors of muscle glycogen phosphorylases and compete with glucose-1-phosphate binding [5]. Vanadate mimics virtually all the biological effects of insulin in a large variety of insulin–dependent tissues [6]. In the study of Nomiya et al. [7] it was shown that streptozotocin (STZ)–induced diabetes mice treated with decavanadate exhibited significant decrease in blood glucose level and decrease of body weight. Among the other insulin mimetic effects on isolated rat adipocytes, vanadate exhibits increasing glucose uptake and inhibition of lipolysis [8].

The aim of this study was to investigate the dose-dependent effect and influence of dissolving agents on insulin-mimetic activity of ammonium decavanadate [$(NH_4)_6V_{10}O_{28} \cdot 6H_2O$), DV]. Two different concentrations (0.1 mM and 1.0 mM) of DV were tested. Influence of solvents on biological activity we investigated using aqueous, isotonic, saline solution (S), and 2 % dimethyl sulfoxide (DMSO). DMSO has the unique capability to penetrate living tissues without damage and this agent is a commonly utilised carrier of chemical compounds that facilitates their incorporation into the cells or tissues. DMSO possesses the antioxidant activity [9] and positive effect in alloxan-induced diabetes in mice [10].

Materials and methods

Insulin-mimetic activity of decavanadate in terms of inhibition of free fatty acids release (IFFAR) and glucose uptake (GU) was tested according the Sakurai et al. [11] and Rodbell [12] *in vitro* experimental model using isolated rat adipocytes pretreated with epinephrine. It could be proposed that the investigated compound has an insulin mimetic activity *in vitro*, if it shows the

same effect as insulin when added to the adipocytes which were pretreated with insulin antagonist.

Chemicals

Ammonium decavanadate, $(NH_4)_6V_{10}O_{28} \cdot H_2O$ (V) was synthesized starting from ammonium metavanadate, NH_4VO_3 (Merck), following the procedure recently reported by Todorovic et al.[13]. The contents of vanadium in the obtained compounds were checked by inductively coupled plasma atomic spectroscopy method (ICP-AES), and no differences were detected in the obtained and the theoretical values.

Animals

Male Wistar rats (six weeks old, weighing 150-180 g) were provided from the Military Medical Academy Animal House, Belgrade. The animals were maintained with commercial rat feed and water available ad libitum. They were housed in polypropylene cages (each group contained ten rats) with a wire mesh top and a hygienic bed of soft wood scrapings with proper humane care. Temperature and humidity were maintained at 25±2 °C and 55±15 %, respectively. Animal studies were conducted in accordance with the institutional guidelines for care and use of laboratory animals.

Preparation of Adipocytes

Rats were sacrificed under ether anesthesia, and the epididymal white adipose tissues were removed and rinsed in isotonic salt solution (0.9 % w/v of NaCl). Up to 1 g of adipose tissue was digested with 10 mg of collagenase Type II (Sigma) in 3 mL, 1 h at 37 °C in Krebs-Ringer bicarbonate (KRB) buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 120 mM $MgSO_4$, 4.75 mM KCl, 1.20 mM KH_2PO_4, 24.0 mM $NaHCO_3$, 2% albumin, pH 7.4). Adipocytes were liberated from the tissue fragments by stirring with a rod, which was manifested by an increased turbidity in the medium. Fragments of tissues still remaining after this treatment were removed with forceps. The suspension of cells was centrifuged for 1 min at 400 x g. The fat cells floated to the surface, and the stromal-vascular cells were sedimented. Stromal-vascular cells were completely removed from the fat cell preparation after three washes. Fat droplets floated more rapidly to the surface than the fat cells and were aspirated from the surface, after gently stirring the cell suspension. The adipocytes were obtained from the pooled adipose tissue of three rats. The washed cells (2×10^6 cells/mL) were suspended in KRB buffer with 5 mM glucose, 0.1 mM/1 mM of decavanadate dissolved in isotonic salt solution (saline) (0.9 % w/v of NaCl) or dimethyl sulfoxide (2 % v/v). Control cells were suspended in KRB buffer with insulin (1 U/mL). Cells were pre-incubated at 37°C for 30 min. After preincubation 10^{-5}M epinephrine dissolved in saline was added, and the cells were incubated at 37°C for 3 h in CO_2 incubator (MMM, Einrichtungen GmbH).

The reaction was stopped by cooling with ice-water. The final concentrations were as follows: glucose 5 mM, decavanadate 0.1mM /1.0 mM, epinephrine 10^{-5} M and saline (0.9 %, v/v)/DMSO (2 %, v/v). The extracellular concentration of free fatty acids was determined by a sensitive colorimetric micro method [14]. The concentration of extracellular glucose was obtained by the GOD/PAP method [15]. All tests were run in triplicate.

The amount of glucose taken up by adipocytes was calculated from the decrease of glucose in the extracellular medium and inhibition of free fatty acids release was calculated from the decrease of free fatty acids in the extracellular medium, too. The glucose uptake (%) and inhibition of free fatty acids release (%) ability of the decavanadate was calculated according to the cells treated with epinephrine: Glucose uptake (%) = 100 – [(c_{DV} / $c_{epinepfrine}$) × 100], c_{DV} and $c_{epinepfrine}$ were the concentration of glucose in the extracellular medium after incubation with DV and epinephrine, respectively. Inhibition of free fatty acid release (%) = 100 – [(c_{DV} / $c_{epinephrine}$) × 100], c_{DV} and $c_{epinepfrine}$ were the concentration of free fatty acids in the extracellular medium after incubation with decavanadate and epinephrine, respectively.

For the easier interpretation of obtained results we calculated the percent of insulin-mimetic effect on isolated adipocytes as the mean values of the both effects, glucose uptake and the inhibition of free fatty acids release:

Insulin-mimetic effect (%) = [Glucose uptake (%) + Inhibition of free fatty acids release (%)] / 2

Statistics

Descriptive statistics was used for the level of glucose and the level of free fatty acids in treated groups and data were shown as mean ± standard error of mean (S.E.M).

Analysis of variance was used to compare insulin-mimetic effect between the treated groups. *Post hoc* comparisons were determined by the least significant difference (LSD) if the *F*-value for the parameters was significant. For statistical analysis, SPSS for Windows 10 was applied and the $P < 0.05$ was considered to be significant.

Results

In order to compare the insulin mimetic activity of the decavanadate according the concentration and solvent we compared inhibition of free fatty acids release and glucose uptake by isolated rat adipocytes treated with epinephrine. Glucose and free fatty acids levels in the extracellular medium of adipocytes treated with DV as well as with insulin were significantly lower than in epinephrine treated cells (Figure 1. a, b).

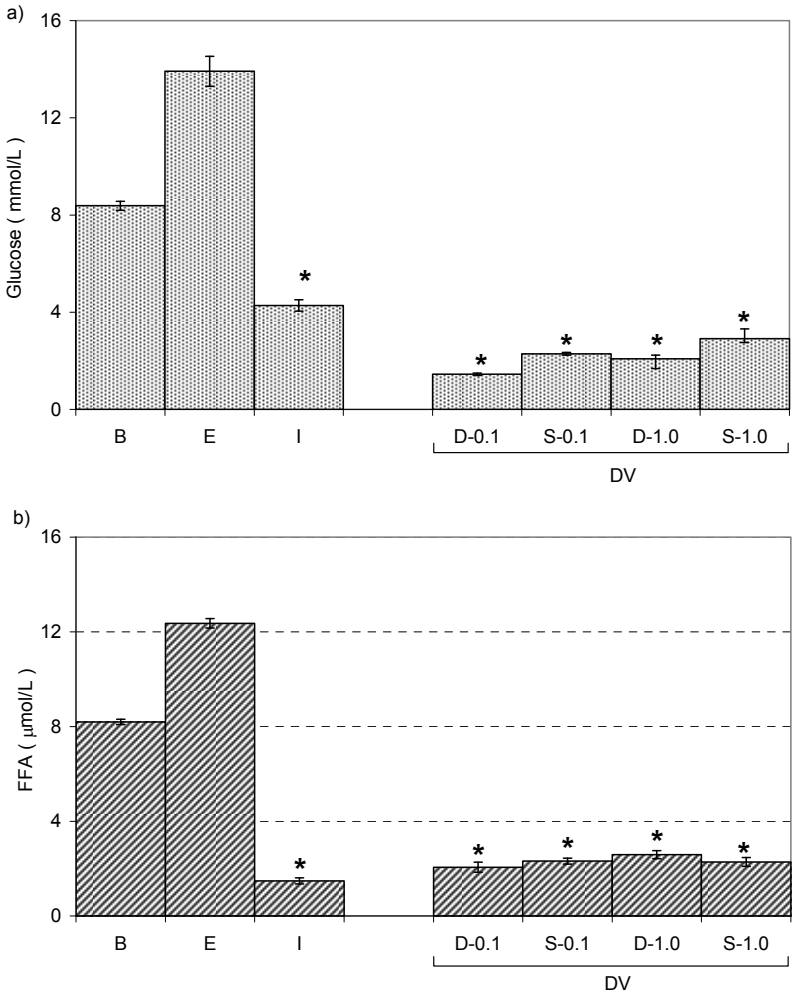

Figure 1. **Effect of ammonium decavanadate (DV) in two different concentrations (0.1 mM, 1.0 mM) and with two different dissolving agents (S-saline, D-DMSO) on concentration of:**
a) glucose, in the extracellular medium
b) free fatty acids in the extracellular medium from isolated rat adipocytes treated with epinephrine in the presence of 5 mM glucose. Each column represents the means ± S.E.M. Blank (B), epinephrine (E) and insulin (I) are adipocytes without any hormone or compound, treated with epinephrine and with insulin, respectively. The insulin- or decavanadate-treated adipocytes compared with epinephrine-treated adipocytes and the level of statistical significance was *$P<0.05$.

Figure 2. shows mean values of the insulin-mimetic effect of DV in two different concentrations (0.1 mM, 1.0 mM) and in two different solvents (saline or DMSO). The biological effect was compared by the *post hoc* test between groups of treated adipocytes.

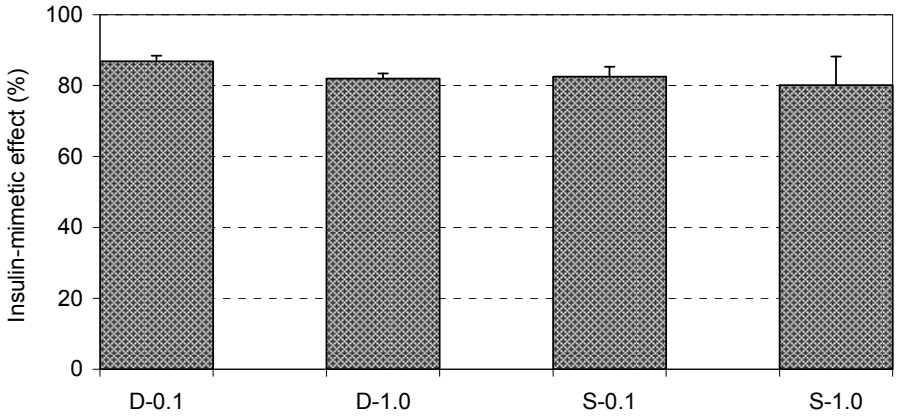

Figure 2. Insulin-mimetic effect (%) of ammonium decavanadate in two different concentrations (0.1 mM, 1.0 mM) and dissolved in DMSO (D) or in saline (S). Data represent the mean ± S.E.M.

In the Table I *post hoc* comparison of insulin mimetic effect between all investigated groups was presented. No differences were detected between investigated groups of treated adipocytes.

Table I Comparisons of the insulin mimetic effect (%) between investigated groups of treated adipocytes (*P* values)

	V-0.1-D (86.83)	V-0.1-S (82.51)	V-1-D (81.98)	V-1-S (80.13)
V-0.1-D	/	0.580	0.691	0.393
V-0.1-S	0.580	/	0.922	0.763
V-1.0-D	0.691	0.922	/	0.713
V-1.0-S	0.393	0.763	0.713	/

Discussion

In this study we investigated insulin mimetic effect of DV on rat adipocytes pretreated with epinephrine. Epinephrine is insulin antagonist. Physiologically, it elevates the blood glucose level by increasing catabolism of glycogen to glucose in the liver, and at the same time begins the breakdown of lipids in fat cells. Epinephrine's actions are mediated through adrenergic receptors. When epinephrine binds to the β-receptor of adipocytes it elevates concentration of cyclic adenosine 3`, 5`-monophosphate (cAMP) by activation of adenylate cyclase. Increase of cAMP subsequently activates some protein kinases. In adipocytes, hormone sensitive lipases are activated to hydrolyze triglycerides to glycerol and free fatty acids. In the group of adipocytes treated only with epinephrine we found elevated glucose level and free fatty acids in extracellular matrix comparing to the untreated adipocytes (Figure 1. a, b). The opposite results were obtained after adding of insulin in epinephrine-pretreated adipocytes. The insulin is the hormone which binds to the insulin receptor and catalyzes the auto-phosphorylation which in turn stimulates the tyrosine kinase which phosphorylates insulin receptor substrate. The signal information is converted to downstream locations such as phosphatidyl inositol-3 kinase, and the glucose transporter-4 which then translocates to the cell membrane. Consequently, glucose uptake by adipocytes is facilitated and releasing of free fatty acids from adipocytes is inhibited, resulting in decreasing of extracellular levels of both glucose and free fatty acids (Figure 1. a, b).

In addition, we investigated the insulin mimetic effect of decavanadate and influence of concentrations and solvents on that bioeffect. Our results showed that decavanadate exhibited high insulin mimetic effect on isolated rat adipocytes. The decavanadate treatment of adipocytes resulted in decreasing of extracellular glucose and free fatty acids levels, which could be explained by facilitated glucose uptake by adipocytes and suppression of lipolysis, respectively, similarly to effect under the insulin treatment. This model is the simple method for evaluating, the *in vitro* insulin mimetic activity of certain compound.

Generally, ammonium decavanadate exhibited insulin mimetic activity in investigated concentrations (0.1 mM and 1.0 mM) and dissolved in both solvents (saline or DMSO). No differences of insulin mimetic effect of DV, expressed in percent (Figure 2 a,b), were detected between adipocytes treated with different concentration of the compound dissolved in saline or DMSO (Table 1).

Although, insulin mimetic effect of decavanadate *in vivo* has been proved [7], *in vitro* investigations are scarce. García-Vicente et al. [16] reported that

sodium decavanadate salt in concentrations ranging from 5 to 50 μM did not stimulate glucose transport into adipose cells *in vitro*.

Our data showed that double higher concentration of decavanadate (0.1 mM = 100 μM) than in their study (50 μM) facilitated glucose uptake by adipocytes. On the basis of our preliminary results should be further investigated narrower range of concentration of decavanadate (between 0.1 mM and 1.0 mM) on insulin mimetic effect.

More attention was directed to other vanadium compounds as insulin mimetics. Therefore, Adachi et al. [17] reported high *in vitro* insulin-mimetic activity of bis(picolinato)vanadyl and bis(6-methylpicolinato) vanadyl complexes, by both uptake of glucose and inhibition of FFA release, in the adipocytes pretreated with epinephrine.

Li et al. [18] showed that vanadate is effective insulin mimetic due its ability to inhibit adipose phosphotyrosine phosphatases (negative regulator of insulin-mediated signal transduction). The same study showed that antilipolysis of vanadate can be manifested via alternative, insulin-independent, signal transduction pathways. Inhibition of phosphotyrosine phosphatases by organo-vanadium compound - bis(maltolato)oxovanadium was proved by recent *in vitro* and *in vivo* studies [19-21].

In the present study we also tested usefulness of DMSO as the well-known carrier of compounds across the cell membrane. It is well known that DMSO is a good polar aprotic solvent that dissolves both polar and nonpolar compounds. Also, it has been documented that DMSO readily crosses most tissue membranes of lower animals and man [22].

Although the study of Wieser at al. [23] showed that DMSO stimulates cAMP accumulation and lipolysis and decrease insulin-stimulated glucose oxidation in free white fat cells of the rat, our data showed that 2 % DMSO as the solvent had no such diabetic effect. Moreover, our data showed high antidiabetic effect of decavanadate dissolved in 2 % DMSO. In this study, we not investigated the insulin mimetic effect of DMSO as the agent on adipocytes. Therefore, we can assume that used concentration of DMSO (2%) was too low for diabetic effect or DMSO facilitated transport of decavanadate in adipocytes so that the overall effect was antidiabetic.

Pharmacological properties of DMSO have been well documented [reviewed in 24]. Even the Layman et al. study [25] showed that oral application of 2 % DMSO reduced development of dietary cholesterol-induced atherosclerosis in rabbits and suppressed the accumulation of cholesterol in tissues and suggested that its antiatherogenic effect was related to its capacity to reduce the binding, uptake, and degradation of plasma low density lipoprotein.

Having in mind that atherosclerosis is chronic complication of diabetes mellitus it is clear that this antiatherogenic agent could be useful in delivering of antidiabetics.

In addition to a number of biological effects which shows decavanadate [26] it is necessary to examine its toxicity in order to safely use its beneficial effects.

In conclusion, on the basis of this *in vitro* experimental study it would be reasonable to continue *in vivo* testing of dose-depending insulin mimetic effect of decavanadate in DMSO.

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Insulin mimetički efekat niskih koncentracija amonijum dekavanadata na izolovane adipocite pacova

Aleksandra Topić[a], Marina Milenković[b], Snežana Uskoković Marković[c], Dragana Vučićević[b]

[a] Institut za medicinsku biohemiju, Farmaceutski fakultet, Univerzitet u Beogradu, Srbija
[b] Institut za mikrobiologiju i imunologiju, Farmaceutski fakultet, Univerzitet u Beogradu, Srbija
[c] Institut za analitičku hemiju, Farmaceutski fakultet, Univerzitet u Beogradu, Srbija

Apstrakt

Ispitana je insulin mimetička aktivnost amonijum dekavanadata (DV), *in vitro* merenjem preuzimanja glukoze (GU) i merenjem inhibicije oslobađanja slobodnih masnih kiselina (IFFAR) na modelu izolovanih belih adipocita Wistar pacova.

Ovaj biološki efekat dekavanadata (u koncentracijama od 0,1 i 1,0 mM) koji je rastvoren u fiziološkom rastvoru (0.9 % NaCl) ili u dimetil sulfoksidu (2% DMSO) ispitan je na adipocitima koji su prethodno tretirani adrenalinom.

Prema dobijenim rezultatima dekavanadat pokazuje snažan insulin mimetički efekat (> 80 %) i to u obe koncentracije i nezavisno od vrste rastvarača.

Na osnovu ovog *in vitro* eksperimenta može se zaključiti da bi bilo opravdano dozno-zavisno ispitivanje insulin mimetičkog efekta dekavanadata u DMSO-u kao rastvaraču u *in vivo* uslovima. Takođe, neophodno je ispitati i toksičnost ovog jedinjenja kako bi se što bezbednije iskoristio njegov insulin mimetički efekat.

Ključne reči: dekavanadat; dimetilsulfoksid; insulin mimetik