

Effects of vanadate on the activities of mice glucokinase and hexokinase

XU Ming-zhi (徐明智)^{†1}, ZHANG Ai-zhen (张爱珍)², LI Xiang-rong (李向荣)³,
XU Wei (许唯)⁴, SHEN Ling-wei (沈凌炜)⁴

¹Department of Endocrinology, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, China)

²Medical Nutrition and Food Hygiene Graduate School, Zhejiang University, Hangzhou 310031, China)

³Division of Life Sciences, City College, Zhejiang University, Hangzhou 310015, China)

⁴Department of Isotopology, Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310009, China)

[†]E-mail: xu921@21cn.com

Received July 14, 2003; revision accepted Feb. 30, 2004

Abstract: This study aimed at acquiring knowledge on the hypoglycemic mechanisms of sodium metavanadate (SMV) showed that the liver glucokinase and muscle hexokinase activities increased rapidly after oral SMV was given, and that the blood glucose level was correlated closely with the activities of the two enzymes but not with the insulin level; which indicated that SMV could improve the altered glucose phosphorylation in diabetic mice independently of stimulating insulin secretion. This was probably one of the mechanisms of hypoglycemic effects of SMV.

Key words: Sodium metavanadate (SMV), Glucokinase, Hexokinase, Blood glucose, Insulin

doi:10.1631/jzus.2004.1245

Document code: A

CLC number: R587.1

INTRODUCTION

Diabetes mellitus is a common and serious disease in the world. Vanadium has been recently confirmed as having insulin-mimetic effects in type 1 and 2 model diabetes, but its mechanism is still not clear (Poucheret *et al.*, 1998). Since vanadium probably has a strong potential to become a good oral anti-diabetic medicine, researching of its hypoglycemic mechanisms maybe helpful to exploit and use this element.

METHODS

Animal preparation

280 female, 28±2 grams, ICR mice were ran-

domly allocated into alloxan group (1% and 200 mg/kg-body weight alloxan) and control group. After 48 hours, the alloxan group mice (whose tail vein blood glucose was large for or equal to 16.67 mmol/L) were confirmed as diabetic mice. The diabetic and control mice were further randomly divided into diabetic D, DV groups, and control C, CV groups. The DV and CV groups drank aqueous solutions (NaCl 80 mmol/L) containing SMV (0.2 mg/ml). The D and C groups drank water-containing NaCl 80 mmol/L only. All mice drank the test solution freely in this study which lasted 5 weeks.

Preparation of specimens

At every weekend up to the 5th week, the chosen (D, *n*=9; DV, *n*=9; C, *n*=6; CV, *n*=6) mice

were killed at 9 o'clock. The orbit blood was collected to assay the blood glucose level by glucose oxidase-peroxidase destination chromometry, and to assay the insulin level by competitive radioimmunoassay.

After the mice died from losing blood, about 1 gram of clear liver and hind-leg muscle were excised, then cut into very small pieces, and put immediately into an ice bath. 4 °C Tris-HCl (0.1 mol/L, pH 7.5), EDTA (5 mmol/L), MgCl₂ (5 mmol/L) and KCl (0.15 mol/L) were added to homogenize the liver and muscle solution to get the clear supernatant fluids.

Assay of liver glucokinase and muscle hexokinase activities

Three ml base solutions were put respectively into high-glucose tube and into low-glucose tube [Tris-HCl (100 mmol/L, pH 7.5), NADP (0.2 mmol/L), ATP (5 mmol/L), MgCl₂ (5 mmol/L), G6PD (0.2 U), 100 mmol/L glucose in high-glucose tube and 0.5 mmol/L glucose in low-glucose tube]. The two tubes were warmed at 30 °C for 5 minutes, and then 20 µl clear liver supernatant fluid was put into them to start the reaction. The increased numeric values of absorption light degree per minute at 340 nm in the spectrum were recorded. The increased values in the low-glucose tube were subtracted from the values in the high-glucose tube. Finally the protein content of the clear liver supernatant fluid was assayed by Lowry Method, and then the liver glucokinase activity was calculated.

The method to assay the muscle hexokinase activity was just like that in the previous procedure. The only difference was that the base solutions contained Tris-HCl (25 mmol/L, pH 7.5), NADP (0.2

mmol/L), ATP (5 mmol/L), MgCl₂ (10 mmol/L), G6PD (0.63 U), 35 mmol/L glucose in the high-glucose tube and 0 mmol/L glucose in the no-glucose tube (Shechter, 1990).

Statistical analysis

Paired-sample *t* test, analysis of variance and correlation analysis were used to analyze the data. Data are reported as $\bar{x} \pm s$. $P \leq 0.05$ was considered significant.

RESULTS

Effects of SMV on glucokinase and hexokinase activities in the experimental mice

At 0 weekend, the two enzymes activities of the DV group were about 5% and 7% of the normal CV group, but markedly increased after the DV group was given SMV for one week ($P < 0.01$), and were up to about 58% and 66% of the control ones; and remained so in the 5 weeks SMV was given to the DV group. The two enzyme activities of CV, C and D groups were not significantly changed after the mice were given the test solutions ($P > 0.05$) (Table 1 and Table 2).

Effects of SMV on blood glucose level in the experimental mice

After being given the SMV solution for one week, the blood glucose of the DV group decreased obviously ($P < 0.01$), and remained so in the 5 weeks SMV was given to the DV group. While the blood glucose levels of CV, C and D groups were not significantly changed ($P > 0.05$) (Table 3).

Table 1 Liver glucokinase activity level of each group (mIU/min/mg protein)

Time (week)	DM		Control	
	DV (n=9)	D (n=9)	CV (n=6)	C (n=6)
0	1.29±0.64	1.33±0.88 [#]	24.56±3.95 ^Δ	24.87±4.77 [♦]
1	15.36±1.57 [*]	1.04±0.45 [#]	26.45±4.02 ^Δ	25.30±2.38 [♦]
2	14.65±2.16 [*]	1.46±0.57 [#]	25.69±3.18 ^Δ	23.94±3.86 [♦]
3	16.49±1.54 [*]	1.18±0.66 [#]	24.09±4.69 ^Δ	25.71±4.48 [♦]
4	15.53±2.26 [*]	1.55±0.73 [#]	26.19±3.54 ^Δ	24.01±3.81 [♦]
5	16.01±1.76 [*]	1.32±0.75 [#]	25.02±3.41 ^Δ	26.45±4.55 [♦]

* $P < 0.01$ vs DV (0 week); [#], ^Δ, [♦] $P > 0.05$

Effects of SMV on insulin level in the experimental mice

In the whole period, the insulin level of the DV, D and C groups were not significantly changed ($P>0.05$), while the insulin level of the CV group was obviously decreased after SMV was given to it for one week ($P<0.01$) (Table 4).

DISCUSSION

Glucose phosphorylation is the first step of glucose metabolism in cell. The procedure is usually

catalyzed by glucokinase in the liver and by hexokinase in other organs or tissues like muscles.

In our study, SMV had remarkable hypoglycemic effects and could obviously enhance the activities of glucokinase and hexokinase, while it had no effect on blood insulin in diabetic mice. Correlation analysis showed that there was obvious negative correlation between the blood glucose level and glucokinase activity ($r=-0.887$, $P<0.0001$) and hexokinase activity ($r=-0.868$, $P<0.0001$), which meant there was obvious positive correlation between the decrease of blood glucose and increase of glucokinase and hexokinase activities.

Table 2 Muscle hexokinase activity level of each group (mIU/min/mg protein)

Time (week)	DM		Control	
	DV (n=9)	D (n=9)	CV (n=6)	C (n=6)
0	1.93±0.50	2.06±0.69 [#]	27.02±5.59 ^Δ	26.82±6.05 [♦]
1	18.62±1.71 [*]	1.86±0.53 [#]	28.17±4.79 ^Δ	25.91±5.81 [♦]
2	17.93±3.49 [*]	1.92±0.86 [#]	26.42±5.87 ^Δ	27.31±4.51 [♦]
3	18.84±2.58 [*]	2.55±1.15 [#]	28.08±5.70 ^Δ	26.87±5.57 [♦]
4	19.25±3.25 [*]	1.73±0.89 [#]	26.92±6.32 ^Δ	28.07±4.78 [♦]
5	18.83±2.83 [*]	2.71±1.94 [#]	27.38±4.86 ^Δ	27.45±6.35 [♦]

* $P<0.01$ vs DV (0 week); ^{#, Δ, ♦} $P>0.05$

Table 3 Blood glucose level of each group (mmol/L)

Time (week)	DM		Control	
	DV (n=9)	D (n=9)	CV (n=6)	C (n=6)
0	18.77±1.28	18.74±1.21 [#]	5.77±0.33 ^Δ	5.91±0.44 [♦]
1	8.94±0.94 [*]	19.96±2.01 [#]	6.01±0.17 ^Δ	5.37±0.38 [♦]
2	9.83±0.22 [*]	17.49±1.47 [#]	5.48±0.35 ^Δ	6.07±0.18 [♦]
3	7.92±0.71 [*]	18.38±1.02 [#]	5.92±0.23 ^Δ	5.45±0.49 [♦]
4	8.09±1.07 [*]	19.15±0.91 [#]	6.27±0.31 ^Δ	5.64±0.26 [♦]
5	9.47±0.93 [*]	18.31±1.84 [#]	5.38±0.51 ^Δ	5.69±0.36 [♦]

* $P<0.01$ vs DV (0 week); ^{#, Δ, ♦} $P>0.05$

Table 4 Insulin level of each group (mU/L)

Time (week)	DM		Control	
	DV (n=9)	D (n=9)	CV (n=6)	C (n=6)
0	6.33±0.86 [*]	5.93±0.95 [#]	29.41±1.74 ^Δ	29.78±1.87 [♦]
1	6.32±1.28 [*]	6.35±0.83 [#]	24.48±2.25 ^Δ	28.53±1.46 [♦]
2	6.31±0.66 [*]	6.01±0.62 [#]	23.84±2.13 ^Δ	29.45±2.35 [♦]
3	7.26±1.02 [*]	6.11±1.19 [#]	24.11±1.98 ^Δ	29.08±0.97 [♦]
4	5.98±0.79 [*]	7.21±0.76 [#]	23.92±1.76 ^Δ	28.46±1.02 [♦]
5	5.49±0.94 [*]	6.32±1.08 [#]	24.61±0.85 ^Δ	29.81±2.33 [♦]

*, #, ♦ $P>0.05$; ^Δ $P<0.01$ vs CV (0 week)

Recently some studies got the same results that many kinds of vanadate could enhance the activities of glucokinase and hexokinase (Carla *et al.*, 2003; Satish *et al.*, 2002; Lucia *et al.*, 2002). Gupta *et al.* (1999) gave sodium orthovanadate (0.6 mg/ml) to alloxan diabetic rats; after 21 days, the activities of the significantly decreased hexokinase isozymes were restored to almost control levels, and using insulin alone also yielded the same result (Gupta *et al.*, 1999).

Some recent investigations on molecule level have demonstrated that vanadate can not only affect insulin receptor level, but can also affect the post-insulin-receptor level. This may possibly provide us some evidence how SMV causes the above-mentioned effects on the glucokinase activity and hexokinase activity.

One of the major effects of vanadate on insulin receptor level is that it can inhibit the action of the protein tyrosine phosphatase (PTPase). For example, vanadate can cause the phosphorylated phosphofructokinase and pyruvate-kinase to be dephosphorylated and thereby strengthen the two enzymes activities by the previous mechanism (Mosseri *et al.*, 2000).

The chief effect of vanadate on the post-insulin-receptor level is the activation of the phosphatidylinositol 3-kinase (PI3-K), an important medium in cellular signal transduction process. For example, Rondinone and Smith (2000) found that vanadate increased the insulin-stimulated upstream signaling including PI3-kinase and PKB activities as well as rate of glucose transport in fat cells from lean and obese Zucker rats of different ages (Rondinone and Smith, 2000).

References

- Carla, R.O., Jose, B.C., Maria, H.M., Suzana, F.Z., Luciana, C.C., Angélica, A., Alessandra, L.G., Vainer, M., Leonardo, F.Z., Licio, A.V., Mario, J.A., 2003. Novel signal transduction pathway for luteinizing hormone and its interaction with insulin: activation of janus kinase/signal transducer and activator of transcription and Phosphoinositol 3-kinase/akt pathways. *Endocrinology*, **144**:638-647.
- Gupta, D., Raju, J., Prakash, J., Baquer, N.Z., 1999. Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats: effect of insulin and vanadate. *Diabetes Research & Clinical Practice*, **46**(1):1-7.
- Lucia, M., Maria, A., Angeles, M., Carmen, A., Fernando, E., 2002. Effects of chronic undernutrition on glucose uptake and glucose transporter proteins in rat heart. *Endocrinology*, **143**:4295-4303.
- Mosseri, R., Waner, T., Shefi, M., Shafrir, E., Meyerovitch, J., 2000. Gluconeogenesis in non-obese diabetic (NOD) mice: in vivo effects of vandadate treatment on hepatic glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. *Metabolism*, **49**(3):321-325.
- Poucheret, P., Verma, S., Grynepas, M.D., McNeill, J.H., 1998. Vanadium and diabetes. *Molecular and Cellular Bio-chemistry*, **188**(1-2):73-80.
- Rondinone, C., Smith, U., 2000. Insulin resistance in fat cells from obese Zucker rats—evidence for an impaired activation and translocation of protein kinase B and glucose transporter 4. *Mol Cell Biochem*, **206**(1-2): 7-16.
- Satish, P., Pamela, A., Graham, R., Stefano, F., Mario, P., Sara, C.K., George, T., Calum, S., 2002. Insulin regulation of insulin-like growth factor-binding protein-1 gene expression is dependent on the mammalian target of rapamycin, but independent of ribosomal S6 kinase activity. *J. Biol. Chem.*, **277**:9889-9895.
- Shechter, Y., 1990. Insulin-mimetic effects of vanadate. Possible implications for future treatment of diabetes. *Diabetes*, **39**(1):1-5.

Welcome visiting our journal website: <http://www.zju.edu.cn/jzus>

Welcome contributions & subscription from all over the world

The editor would welcome your view or comments on any item in the journal, or related matters

Please write to: Helen Zhang, Managing Editor of JZUS

E-mail: jzus@zju.edu.cn Tel/Fax: 86-571-87952276