Comparative study of insulin-mimetic activity of vanadium and zinc complexes

Yusuke Adachi and Hiromu Sakurai

Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

Abstract In recent years, some metal ions and their complexes have been found to treat diabetes mellitus (DM) in not only experimental animals but also humans. Among them, vanadyl(IV) and zinc(II) ions as well as their complexes have been shown to be the most expectant agents. Then, the differences in the insulin-mimetic effects of vanadyl(IV) and zinc(II) ions as well as their complexes were investigated. *In vitro* insulin-mimetic activities of the compounds were estimated by both glucose uptake enhancing effect and inhibitory effect of free fatty acid (FFA) release in isolated rat adipocytes treated with epinephrine. Vanadyl(IV) compounds exhibited the higher glucose uptake enhancing activities than zinc(II) compounds, however, vanadyl(IV) compounds showed the lower inhibitory activities of FFA release than zinc(II) compounds. Next, we examined the blood glucose lowering effects of the compounds in both streptozotocin (STZ)-induced type 1 DM mice and type 2 DM KK-A^y mice by intraperitoneal injection of the compounds. Vanadyl(IV) compounds lowered the high blood glucose levels in both types of DM mice. In contrast, zinc(II) showed the blood glucose lowering effects in KK-A^y mice.

Key word: vanadyl(IV); zinc(II); insulin-mimetic effect; type 1 diabetes mellitus; type 2 diabetes mellitus

Introduction

The number of patients suffering from diabetes mellitus (DM), which develops many secondary complications, was reported to increase in approximately 173 million people in the world in 2002. Although several types of insulin preparations for type 1 DM and those of synthetic drugs for type 2 DM have been developed and clinically used, they have several problems such as physical and mental pain due to daily insulin injections and some serious side effects, respectively. To improve the quality of life (QOL) of diabetic patients, the development of new type therapeutic agents that are orally active and free from side effects, for DM is an urgent need.

Recently, metal ions such as selenium, manganese, tungsten, vanadium and zinc have been proposed as candidates for treating DM [1-5]. To specify potent metal ions as antidiabetic agents, we tested their insulin-mimetic activities by using isolated rat adipocytes treated with epinephrine. Among them, vanadyl(IV) (+4 oxidative state of vanadium) and zinc(II) were found to have high insulin-mimetic activities and were thought to be less toxic than other metal ions [6]. From these observations, we focused on vanadyl(IV) and zinc(II) complexes, and found some potent insulin-mimetic vanadyl(IV) and zinc(II) complexes, which had higher activities than their ions [6-7]. In this paper, we compared the insulin-mimetic activities of $VOSO_4$, and $ZnSO_4$ (or $ZnCl_2$), as well as their complexes, bis(maltolato)-oxovanadium(IV) ($VO(ma)_2$) and bis(maltolato)zinc(II) ($Zn(ma)_2$), which were already found to have high insulin-mimetic activities in DM animals [8, 9].

Corresponding author: Hiromu Sakurai
Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

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Experimental methods

1. Materials

Vanadyl sulfate ($VOSO_4 \cdot 2.8H_2O$), zinc sulfate ($ZnSO_4 \cdot 7.0H_2O$), zinc chloride ($ZnCl_2$), and maltol (3-hydroxy-2-methyl-4-pyrone) were purchased from Wako Pure Chemical Co. (Osaka, Japan). Bis(maltolato)-oxovanadium(IV) ($VO(ma)_2$) and bis(maltolato)zinc(II) ($Zn(ma)_2$) complexes were prepared as reported [8,9].

2. Animals

Male Wistar rats (7 weeks old) and ddY mice (7 weeks old) were obtained from Shimizu Experimental Material Co. (Kyoto, Japan). Male KK-A^y mice (4 weeks old) with type 2 DM were purchased from CLEA Japan, Inc. (Tokyo, Japan). KK-A^y mice were used for *in vivo* study when they were 12 weeks old. STZ-mice were prepared as follows: Male ddY mice (7 weeks old) received intraperitoneal (*i.p.*) injections

of STZ (two times of 100 mg/kg body weight at interval of seven days).

3. *In vitro* insulin-mimetic activities of vanadyl(IV) and zinc(II) compounds in isolated rat adipocytes treated with epinephrine

In vitro insulin-mimetic activities of vanadyl(IV) and zinc(II) compounds were determined by both the enhancing effect of glucose uptake and the inhibitory effect of free fatty acid (FFA) release in isolated rat adipocytes treated with epinephrine [10,11]. On the outer solution of the cells, FFA and glucose levels were determined by using a FFA kit (NEFA C-test Wako; Wako Pure Chemicals, Osaka, Japan) and an automatic glucose analyzer (Fuji Dry Chem; Fuji Medical Co., Tokyo, Japan), respectively. The glucose uptake levels were evaluated according to the decrease in glucose concentration in the medium [11].

4. Blood glucose lowering effects of vanadyl(IV) and zinc(II) compounds in type 1 and type 2 diabetic mice.

STZ mice with type 1 DM received *i.p.* injections of $VOSO_4$ (single injection of 20 mg (196 μmol) V/kg body weight), $ZnSO_4$ (single injection of 10 mg (153 μmol) Zn/kg body weight), $VO(ma)_2$ (single injection of 5 mg (98 μmol) V/kg body weight) or $Zn(ma)_2$ (single injection of 10 mg (153 μmol) Zn/kg body weight). The KK-A^y mice with type 2 DM were treated by daily *i.p.* injections of $VOSO_4$ (2.5 mg (49 μmol) V/kg body weight for 14 days), $ZnCl_2$ (3 mg (46 μmol) Zn/kg body weight for first seven days and then 5 mg (76 μmol) Zn/kg body weight for eight days), $VO(ma)_2$ (1 mg (20 μmol) V/kg body weight at the first day and then 0.1 mg (2 μmol) V/kg body weight for 13 days.) or $Zn(ma)_2$ (4.5 mg (69 μmol) Zn/kg body weight for the first two days and then adjusted to approximately 2-4.5 mg (31-69 μmol) V/kg body weight according to the blood glucose level for 12 days). Blood samples were obtained from tail vein of mice, and blood glucose levels were measured by using a glucose oxidase method (Glucocard; Arkray, Kyoto, Japan).

Results

1. *In vitro* insulin-mimetic activities of vanadyl(IV) and zinc(II) compounds

The concentration-dependent enhancing effects of glucose uptake and the inhibitory effects of FFA release were observed in all compounds (data are not shown), and the maximal glucose uptake levels and inhibitory effects of FFA release (%) were calculated from these data. The maximal glucose uptake levels of vanadyl(IV) compounds were significantly higher than those of zinc(II) compounds (Fig. 1, left). In addition, the inhibitory effects of FFA release of vanadyl(IV) compounds were significantly lower than those of zinc(II) compounds (Fig. 1, right).

2. Blood glucose lowering effects of vanadyl(IV) and zinc(II) compounds in diabetic mice

Blood glucose lowering effects of vanadyl(IV) and zinc(II) compounds were examined in type 1 DM STZ-mice and type 2 DM KK-A^y mice. When $VO(ma)_2$ was given by single *i.p.* injection at 5 mg (98 μmol) V/kg body weight, the blood glucose levels were lowered to the range between 100-200 mg/dL within 48 hours after the administration (Fig. 2, left). The lowering effects of $VO(ma)_2$ continued for at least 4 days after the administration (data are not shown). Also, $VOSO_4$ showed the blood glucose lowering effects in STZ-mice by *i.p.* injections at 20 mg (393 μmol) V/kg body weight. However, both zinc(II) compounds showed no blood glucose lowering effects in type 1 diabetic STZ-mice. On the other hand, $VO(ma)_2$, $ZnCl_2$, and $Zn(ma)_2$ significantly lowered the high blood glucose levels in type 2 diabetic KK-A^y mice by daily *i.p.* injections for 14 days (Fig. 2, right). While $VOSO_4$ showed no blood glucose lowering effects in KK-A^y mice by *i.p.* injections for 14 days at 2.5 mg (49 μmol) V/kg body weight, because those doses were relatively lower than other compounds. From these results,

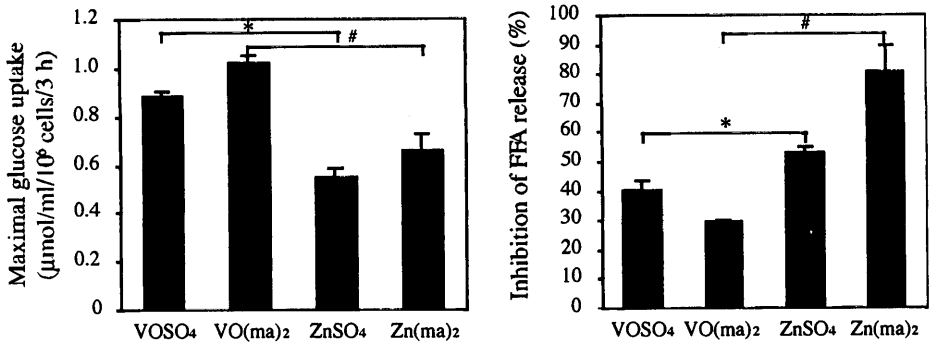

Fig. 1. Maximal glucose uptake level (left) and inhibition of FFA release at 500 μM (right) in the isolated rat adipocytes treated with $VOSO_4$, $VO(ma)_2$, $ZnSO_4$ or Zn(ma) for 3 h incubation. Data are expressed as the means ± SDs for 3 experiments. *Significance at $P < 0.01$ vs. $VOSO_4$, #Significance at $P < 0.01$ vs. $VO(ma)_2$.

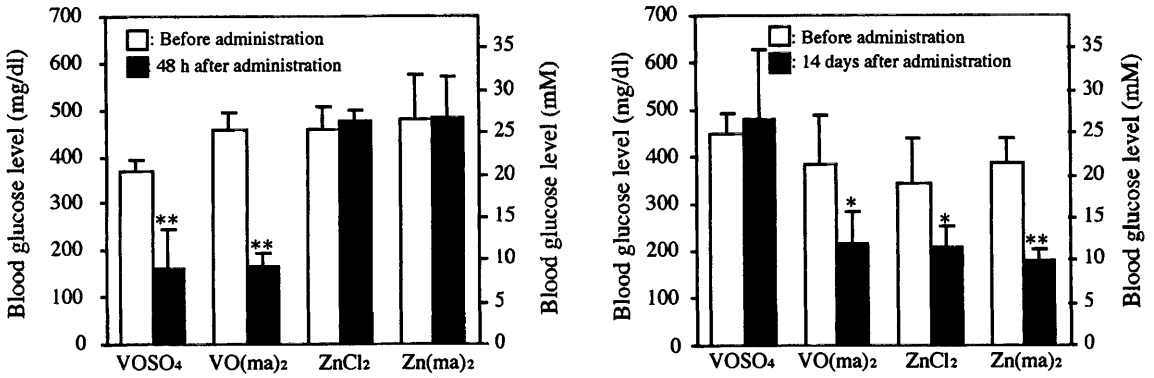

Fig. 2 Changes of blood glucose levels in diabetic mice treated with $VOSO_4$, $VO(ma)_2$, $ZnCl_2$ or $Zn(ma)_2$. Type 1 diabetic STZ-mice were examined for acute effects of compounds (left), and type 2 diabetic KK-A_y mice were examined for chronic effects of compounds (right). The doses of compounds are described in experimental methods. Data are expressed as the means ± SDs for 4-6 mice. *Significance at $P < 0.05$ vs. blood glucose level before administration. **Significance at $P < 0.01$ vs. blood glucose level before administration.

vanadyl(IV) complex, $VO(ma)_2$ was found to have the blood glucose lowering effects in both types of DM, however, zinc(II) compounds were effective only in type 2 DM.

Discussion

Epinephrine activates the adenylate cyclase, which causes to transform ATP to cyclic adenosin 3',5'-monophosphate (cAMP) through the β-receptor of adipocytes, in turn activates lipase. The activated lipase then activates to hydrolyze triglycerides to free fatty acids (FFA), which will be released outside of the cells [12]. The binding of insulin to the insulin receptor increases glucose uptake in adipocytes by stimulating the translocation of the glucose transporter (GLUT-4) from intracellular sites to the plasma membrane thorough activations of tyrosine phosphorylation, insulin receptor substrate (IRS) and phosphatidyl inositol-3-kinase (PI3-K) in insulin signal pathway. These reactions subsequently cause to activate phosphodiesterase (PDE), which transforms cAMP to 5'-AMP in cells. Consequently, glucose uptake is enhanced and epinephrine-induced FFA release is subsequently inhibited by insulin in isolated rat adipocytes [12,13]. We have recently proposed a possible mechanism, by which vanadyl(IV) and zinc(II) act at sites such as PI3-K, glucose transporter, and PDE in cells to exhibit glucose uptake enhancing effects and inhibitory effects of FFA release in isolated rat adipocytes treated with epinephrine [6,14]. However, the order of glucose uptake enhancing activities of vanadyl(IV) and zinc(II) compounds was disagree with that of inhibitory effects of FFA release (Fig. 1). These results suggest that the action sites of vanadyl(IV) and zinc(II) in the cells partially differ each other.

Also, the different effects of vanadyl(IV) and zinc(II) compounds were observed in type 1 diabetic STZ-mice and type 2 diabetic KK-A^y mice. $VO(ma)_2$ exhibited the blood glucose lowering effects in both types of DM mice (Fig. 2). Interestingly, zinc(II) compounds showed the blood glucose lowering effects only in type 2 DM KK-A^y mice. It is thought that the blood glucose lowering effects by metal compounds in type 1 diabetic STZ mice depend on their glucose-uptake enhancing effects in cells, because type 1 DM are an absolute lack of insulin. Therefore, zinc(II) compounds may exhibit no blood glucose lowering effects in type 1 diabetic STZ mice because of low glucose-uptake enhancing activities in adipocytes (Fig. 1). However, some authors reported the blood glucose lowering effects by zinc(II) in type 1 DM animals [15,16]. It is necessary to examine the effects in other types of DM model animals.

In conclusion, the different mechanism for the insulin-mimetic effects of vanadyl(IV) and zinc(II) complexes was indicated in *in vitro* evaluations. Vanadyl(IV) complex, $VO(ma)_2$ was confirmed to treat not only type 1 DM but also type 2 DM, while zinc(II) complex improved type 2 DM. More studies on both vanadyl(IV) and zinc(II) complexes are needed for clinical use in the future.

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