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# Improvement of hyperglycaemia and metabolic syndromes in type 2 diabetic KKA$^{y}$ mice by oral treatment with [meso-tetrakis(4-sulfonatophenyl) porphyrinato]oxovanadium(IV)(4–) complex

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## Abstract

Recently, we reported that [meso-tetrakis(4-sulfonatophenyl)porphyrinato]oxovanadium(IV)(4–), VO(tpps), shows in-vitro insulin-mimetic and in-vivo anti-diabetic activity in streptozotocin (STZ)-induced type 1 diabetic mice. This result prompted us to examine its ability in type 2 diabetic model KKA$^{y}$ mice with insulin resistance. We studied the in-vivo anti-diabetic activity of VO(tpps), compared with that of vanadium(IV) oxide sulfate, VS, as control. Both compounds were orally administered at doses of 5–10 mg (0.1–0.2 mmol) V/kg body weight to the KKA$^{y}$ mice for 28 days. VO(tpps) normalized the hyperglycaemia within 15 days, while VS lowered the blood glucose concentration only by a small degree. In addition, metabolic syndromes characterized by insulin and leptin resistance were significantly improved in VO(tpps)-treated KKA$^{y}$ mice compared with those treated with VS. The improvement in diabetes was validated by oral glucose tolerance test and decrease in $HbA_{1c}$ concentration. Based on these observations, VO(tpps) is proposed to be an orally active oxovanadium(IV)–porphyrin complex for treating not only type 2 diabetes but also metabolic syndromes in animals.

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## Introduction

The number of patients suffering from type 2 diabetes mellitus is rapidly reaching epidemic levels in several countries. Type 2 diabetes mellitus is clinically characterized by insulin resistance in target tissues, including the adipose tissue, skeletal muscle and liver, and by defects in insulin secretion from pancreatic $\beta$-cells (Martin et al 1992; Vague & Raccah 1992; Reaven 1993; Taylor et al 1994). Insulin resistance, which is associated with dyslipidaemia, arterial hypertension and atherosclerotic cardiovascular diseases, has been implicated in the pathogenesis of the long-term complications of type 2 diabetes mellitus (DeFronzo & Ferrannini 1991). Therefore, treatments that not only lower the blood glucose concentration but also improve both insulin and leptin resistance are clearly in high demand compared with the commonly used therapies of sulfonylurea and other drugs (Groop 1992; Simpson et al 2006). Leptin, which is primarily synthesized and secreted from white adipose tissue, is a hormonal protein that regulates glucose metabolism and insulin sensitivity via leptin receptors in the brain and peripheral tissues (Kieffer & Habener 2000; Spiegelman & Flier 2001; Ceddia et al 2002). Therefore, it is recognized that improvement of the reduced insulin and leptin sensitivity treats not only diabetes but also metabolic syndromes such as obesity, dyslipidaemia, arterial hypertension and atherosclerotic cardiovascular diseases.

It has been established that vanadium compounds exert an insulin-mimetic action in both in-vitro and in-vivo systems, including their ability to improve glucose homoeostasis and insulin resistance in animal models of diabetes mellitus (Cam et al 1999; Reul et al 1999; Thompson et al 1999; Sakurai 2002; Sakurai et al 2002, 2003; Rehder 2003; Crans et al 2004; Srivastava & Mehdi 2005). In recent years, several reports have documented vanadium therapy, which induces improvement of insulin sensitivity in the liver and muscles of type 2 diabetic subjects (Goldfine et al 1995, 2000; Cusi et al 2001).

Since the discovery of the orally active insulin-mimetic bis(6-methylpicolinato)oxovanadium(IV) complex, $VO(6mpa)_2$, for the treatment of KKA$^{y}$ mice, a type 2 diabetic mouse

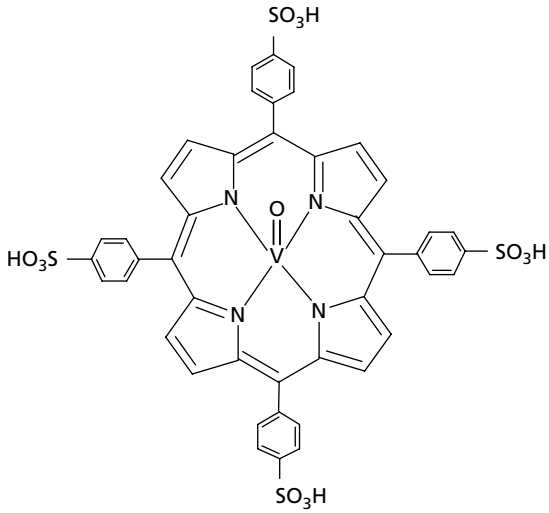


**Figure 1** Structure of VO(tpps).

model, in 1999 (Fujisawa & Sakurai 1999), the therapeutic potential of oxovanadium(IV) complexes has been of great interest. We, and other researchers, have developed several types of oxovanadium(IV) complexes with different coordination environments, such as $VO(N_2O_2)$, $VO(O_4)$ and $VO(S_2O_2)$ (Yuen et al 1999; Takeshita et al 2001; Sakurai 2002; Sakurai et al 2002, 2003; Sasagawa et al 2002), for the treatment of type 2 diabetes mellitus in animals. However, only a few complexes that have a $VO(N_4)$ coordination environment, such as bis(biguanidato)oxovanadium(IV) $(VO(big)_2)$, bis(*N′, N′*-dimethylbiguanidato)oxovanadium(IV) $(VO(metf)_2)$ and bis(*β*-phenethylbiguanidato)oxovanadium(IV) $(VO(phenb)_2)$, have been prepared (Woo et al 1999). Among them, $VO(metf)_2$ was found to lower the blood glucose concentration in streptozotocin (STZ)-induced diabetic rats (STZ-rats), a type 1 diabetic model, when administered by acute oral administration.

Recently, we found that [meso-tetrakis(1-methylpyridinium-4-yl)porphyrinato]oxovanadium(IV)(4+) (VO(tmpyp)), which has the $VO(N_4)$ coordination environment, is a potential insulin-mimetic oxovanadium(IV)–porphyrin complex for treating STZ-rats when injected intraperitoneally together with sodium ascorbate (Sakurai et al 2004). This finding prompted us to develop more active oxovanadium(IV)–porphyrin complexes. Our previous confirmation of in-vivo anti-diabetic activity of [meso-tetrakis(4-sulfonatophenyl)porphyrinato]oxovanadium (IV)(4–) (VO(tpps)) (Figure 1) after long-term administration in STZ-mice (Saha et al 2005, 2006) has suggested a possibility for estimating its hypoglycaemic ability and improvements of insulin and leptin resistance in type 2 diabetic animals. Here, we report the anti-diabetic activity and improvement of insulin and leptin resistance of VO(tpps) when it was orally administered to type 2 diabetic $KKA^y$ mice for 28 days.

## Materials and Methods

### Materials

The ligand meso-tetrakis(4-sulfonatophenyl)porphyrin ($H_2$tpps) was purchased from Frontier Scientific, Inc. (Logan, UT). Vanadium(IV) oxide sulfate n-hydrate ($VOSO_4 \cdot nH_2O$), obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan), was standardized complexometrically with EDTA and ascertained to be a trihydrate (VS). Sephadex LH-20 was obtained from Amersham Biosciences KK (Tokyo, Japan). All other reagents and solvents were commercially available and of the highest grade of purity and were therefore used without further purification.

VO(tpps) was prepared as reported (Saha et al 2006). Briefly, $H_2$tpps (0.2 g, 0.2 mmol) and $VOSO_4 \cdot 3H_2O$ (0.86 g, 3.99 mmol) were refluxed in N,N-dimethylformamide (50 mL) on a stirring hot plate at 150°C for 24 h. The crude material was purified by gel chromatography (Sephadex LH-20; eluent: $H_2O$). Finally, the aqueous solution was concentrated and dried under high vacuum. The structure of VO(tpps) (Figure 1) was characterized by elemental analyses, mass spectrometry and IR, visible, and ESR spectra (Table 1).

### Animals

Male non-diabetic C57BL/6J mice, 5 weeks old, 17–19 g, and male $KKA^y$ mice, 5 weeks old, 25–27 g, were obtained from CLEA Japan, Inc. (Osaka, Japan) and were used for in-vivo study when they were 12 weeks old. $KKA^y$ mice, obtained by crossing between glucose-intolerant black KK female mice and yellow male obese $A^y$ mice, are characterized by hyperphagia due to leptin resistance, followed by obesity, and development of hyperleptinaemia, hyperinsulinaemia, diabetes, dyslipidaemia and hypertension after approximately 8 weeks of age (Iwatsuka et al 1970; Taketomi et al 1975; Chang et al 1986). Therefore, $KKA^y$ mice are known to serve as an excellent model that closely resembles obesity-linked type 2 diabetes in man, expressing several disorders within a single individual. C57BL/6J mice were generally used as a non-diabetic control for $KKA^y$ mice. All mice were individually housed in a small cage and maintained on a 12-h light–dark cycle in a temperature-controlled animal room. All mice were allowed free access to solid food (MF; Oriental Yeast Co., Tokyo, Japan) and tap water. All animal experiments were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University (KPU) and performed according to the guidelines for animal experimentation developed by KPU.

### In-vivo evaluation in $KKA^y$ mice

The mice were divided into the following 5 groups: Group I, non-diabetic C57BL/6J mice (n = 5) that were orally administered saline alone; Group II, control $KKA^y$ mice (n = 5) that were orally administered saline alone; Group III, $KKA^y$ mice (n = 5) that were administered vanadium(IV) oxide sulfate (VS) dissolved in saline orally; Group IV, $KKA^y$ mice (n = 7) that were administered VO(tpps) dissolved in saline orally; and Group V, $KKA^y$ mice (n = 6) that were administered $H_2$tpps dissolved in saline orally. VS and VO(tpps) dissolved in saline were administered to the mice of Groups III and IV, respectively, at doses of 5 mg (0.1 mmol) V/kg body weight for the first 5 days and then 10 mg (0.2 mmol) V/kg body weight for the following 23 days. The $H_2$tpps dissolved in saline was administered to the mice of Group V at doses

**Table 1** Physicochemical properties of VO(tpps)

| Complex | IR spectrum in KBr $\nu_{V=O}$ ($cm^{-1}$) | FAB$^-$ (m/z) | Elemental analysis Calcd (Found) | | |
|---|---|---|---|---|---|
| | | | C% | H% | N% |
| $C_{44}H_{28}O_{12}S_4N_4VO\bullet 8H_2O\bullet 2C_3H_7NO$(VO(tpps)) | 1005 | 998 | 46.55 (46.21) | 4.53 (4.48) | 6.51 (6.55) |

| Complex | Visible absorption spectrum | | | Solvent | ESR parameter | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | g-value | | | A-value ($10^{-4}$ $cm^{-1}$) | | |
| | $\lambda_1$ nm ($\varepsilon$)[a] | $\lambda_2$ nm ($\varepsilon$)[a] | $\lambda_3$ nm ($\varepsilon$)[a] | | $g_0$ | $g_{//}$ | $g_\perp$ | $A_0$ | $A_{//}$ | $A_\perp$ |
| VO(tpps) | 436 (219.5) | 564 (18.8) | 604 (7.3) | $H_2O$ | 1.967 | 1.938 | 1.982 | 106 | 183 | 67 |

[a] $10^3$ M$^{-1}$ $cm^{-1}$.

corresponding to the equimolar concentrations of VO(tpps). These compounds were administered to each mouse for 28 days at about 1100 h after the determination of their blood glucose concentrations. The blood sample required for the daily analysis of glucose concentration was collected from the tail vein of each mouse, and the blood glucose concentration was measured using a GLUCOCARD (ARKRAY Inc., Kyoto, Japan). The body weight of mice was measured daily before the administration of the saline and vanadium(IV) compounds. The intake of solid food and drinking water in each mouse was measured daily throughout the experiment before the administration of the saline and vanadium(IV) compounds.

After the oral administration of saline alone, VS, VO(tpps) or $H_2$tpps for 28 days, blood samples were collected by orbital exsanguination from the mice anaesthetized with ether and were centrifuged at 5000 rev $min^{-1}$ for 10 min at 4°C. The serum samples were separated and subjected to analyses of urea nitrogen (UN), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), triglycerides (TG), total cholesterol (TCHO), free fatty acids (FFA), insulin and leptin concentrations. The serum UN, GPT, GOT, TG and TCHO concentrations were estimated by using a FUJI DRY-CHEM analyzer (FUJIFILM Medical Co. Ltd, Tokyo, Japan). The NEFA C test and Glazyme insulin-EIA test (Wako Pure Chemical Industries Ltd.) were used to determine the serum FFA and insulin concentrations, respectively. The serum leptin concentration was measured using a Quantikine Mouse Leptin Immunoassay kit from R&D Systems, Inc. (Minneapolis, MN). The glycosylated haemoglobin ($HbA_{1c}$) concentration in the blood was measured using a DCA 2000 analyzer (Bayer Corp., Tokyo, Japan).

### Oral glucose tolerance test

After the administration of saline alone, VS, VO(tpps) or $H_2$tpps for 28 days, oral glucose tolerance tests (OGTT) were performed. All mice were fasted for 12 h, and glucose at a dose of 1 g (kg body weight)$^{-1}$ was orally administered. Blood samples were collected from the tail veins at 0, 15, 30, 45, 60, 90, 120 and 180 min after the glucose administration. The blood glucose concentrations were measured using the GLUCOCARD as described.

### Statistical analysis

All experimental results are expressed as the means ± standard deviation (s.d.). Statistical analysis was performed using one-way analysis of variance followed by Tukey–Kramer's multiple-comparison post-hoc tests. Differences were considered to be statistically significant when $P < 0.01$ or $< 0.05$.

## Results and Discussion

### Blood glucose normalization

Previously, we found that VO(tpps) is a potent orally active insulin-mimetic oxovanadium(IV)–porphyrin complex for treating STZ-mice (Saha et al 2005, 2006). We then examined the anti-diabetic effect of VO(tpps) in KKA$^y$ mice upon oral administration for 28 days and compared it with that of the control VS. Figure 2 illustrates the changes in the blood glucose concentrations in saline-treated non-diabetic C57BL/6J mice (Group I) and KKA$^y$ mice treated with saline alone (Group II), VS (Group III) and VO(tpps) (Group IV) at doses in the range 5–10 mg (0.1–0.2 mmol) V/kg body weight, and $H_2$tpps (Group V) at doses corresponding to equimolar concentrations of VO(tpps). The blood glucose concentration in saline-treated KKA$^y$ mice (Group II) was significantly higher compared with that in saline-treated non-diabetic C57BL/6J mice (Group I) throughout the study. When VS was administered at a dose of 5 mg (0.1 mmol) V/kg body weight for the first 5 days, the blood glucose concentration in KKA$^y$ mice (Group III) was not lowered. When the dose was increased to 10 mg (0.2 mmol) V/kg body weight for the following 23 days, the blood glucose concentration was not significantly lowered, compared with that in saline-treated KKA$^y$ mice. On the other hand, when VO(tpps) was administered at a dose of 5 mg (0.1 mmol) V/kg body weight, the blood glucose concentration in KKA$^y$ mice (Group IV) was rapidly lowered after 2 days. The same dosage of the complex was then maintained for the following 3 days. However, the blood glucose concentration remained at approximately 350 mg dL$^{-1}$ (19.4 mM) for the first 5 days. The dose of the complex was then increased to 10 mg (0.2 mmol) V/kg body weight for the following 23 days. After such adjustment, the blood glucose concentration gradually lowered and remained at approximately

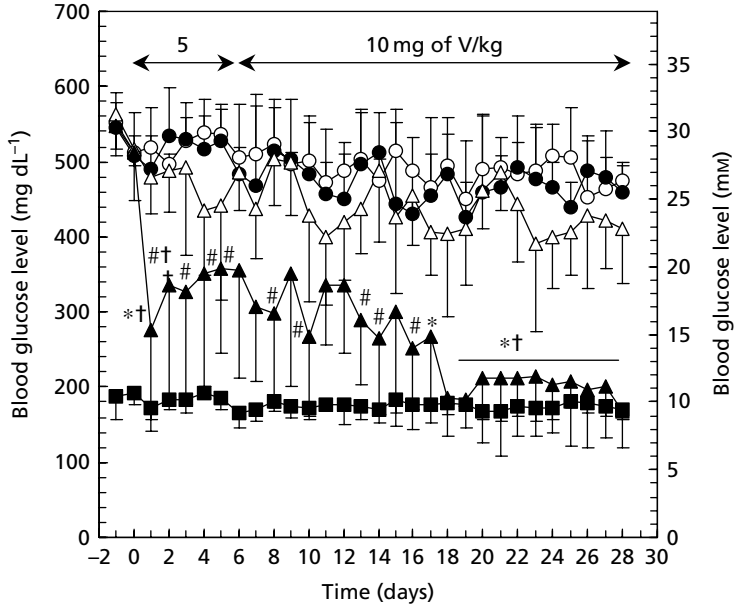


**Figure 2** Changes in blood glucose concentration in saline-treated non-diabetic C57BL/6J mice (closed squares, n = 5) and type 2 diabetic KKA$^y$ mice after oral administration for 28 days of saline (open circles, n = 5), VS (open triangles, n = 5) and VO(tpps) (closed triangles, n = 7) at doses of 5–10 mg (0.1–0.2 mmol) V/kg body weight, and $H_2$tpps (closed circles, n = 6) at doses corresponding to an equimolar concentration of VO(tpps). VS, VO(tpps) and $H_2$tpps were dissolved in saline. *$P < 0.01$, #$P < 0.05$ vs saline-treated KKA$^y$ mice; †$P < 0.01$, vs VS-treated KKA$^y$ mice.

200 mg dL$^{-1}$ (10 mM) for the last 11 days; this value was close to the blood glucose concentration in non-diabetic C57BL/6J mice (Figure 2). There was no significant difference between the blood glucose concentrations of VO(tpps)-treated KKA$^y$ mice and C57BL/6J mice for the last 10 days. $H_2$tpps did not show any hypoglycaemic effects at doses that were equivalent to those of VO(tpps) (Figure 2). Reportedly, the blood glucose concentration remained approximately 300 mg dL$^{-1}$ (16.7 mM) in KKA$^y$ mice after oral treatment with VO(6mpa)$_2$ at a dose of 5–10 mg (0.1–0.2 mmol) V/kg body weight for 14 days (Fujisawa & Sakurai 1999). These results demonstrated that chronic oral administration of VO(tpps) exhibits a higher hypoglycaemic effect in KKA$^y$ mice than VS and VO(6mpa).

### Changes of food and water intakes and body weight

The daily food intake in saline-treated KKA$^y$ mice was significantly higher compared with that in C57BL/6J mice (Figure 3A). On the other hand, the daily food intake was low in VS- and VO(tpps)-treated mice compared with that in saline-treated KKA$^y$ mice after 1 or 2 days and remained constant throughout the experiment. There was no significant difference in the daily food consumption between C57BL/6J mice and KKA$^y$ mice treated with VO(tpps). Water intake in VS- and VO(tpps)-treated mice was significantly reduced compared with that in saline-treated KKA$^y$ mice after 1 day and remained almost constant throughout the experiment (Figure 3B).

Daily changes in body weight in each group of mice are shown in Figure 4. There were no significant changes between the initial and final body weight of C57BL/6J and KKA$^y$ mice. The initial body weight was similar among the 4 groups of KKA$^y$ mice; however, it tended to be low in non-diabetic C57BL/6J mice. After the 28 days of treatment, the gain in body weight was similar in all groups. As administration of VO(tpps) complex in type 2 diabetic KKA$^y$ mice resulted in lowering of the blood glucose concentration without causing loss of body weight, it was assumed that hypoglycaemia was not modified by food and water intake but was controlled by VO(tpps) treatment.

### Oral glucose tolerance test

To evaluate the improvement in the glucose tolerance ability in KKA$^y$ mice, an OGTT was performed after the 28 days of treatment. After oral administration of glucose, the blood glucose concentration of saline-treated KKA$^y$ mice increased to a maximal concentration after 15 min and then decreased gradually. Similar phenomena were observed in the cases of VS- and $H_2$tpps-treated KKA$^y$ mice. However, the elevation of the blood glucose concentration in KKA$^y$ mice treated with VO(tpps) was significantly suppressed compared with that in saline-treated and VS-treated KKA$^y$ mice (Figure 5). These results demonstrate that the impaired glucose intolerance in KKA$^y$ mice is ameliorated by VO(tpps) treatment.

### Serum parameters

The concentrations of $HbA_{1c}$, UN, GPT, GOT, TG, TCHO, FFA, insulin and leptin in the serum of C57BL/6J and KKA$^y$ mice are summarized in Table 2. $HbA_{1c}$ concentration in VO(tpps)-treated KKA$^y$ mice decreased significantly compared with that in saline-treated KKA$^y$ mice. On the other hand, VS moderately improved $HbA_{1c}$ concentration in

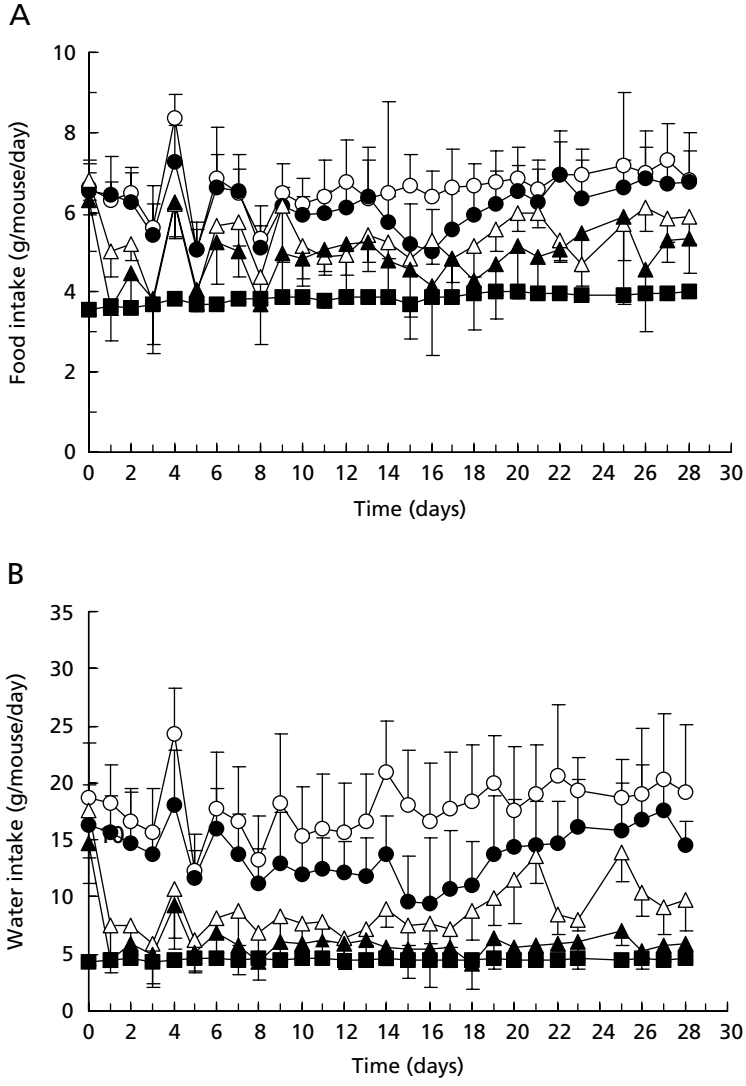


**Figure 3** Changes of food intake (A) and water intake (B) in saline-treated non-diabetic C57BL/6J mice (closed squares, n = 5) and type 2 diabetic KKA$^y$ mice after oral administration of saline (open circles, n = 5), VS (open triangles, n = 5), VO(tpps) (closed triangles, n = 7) and $H_2$tpps (closed circles, n = 6).

KKA$^y$ mice, while $H_2$tpps had no effect. Similar results were observed when STZ-mice received either VO(tpps) at doses of 4–10 mg (0.08–0.2 mmol) V/kg body weight ($HbA_{1c} = 6.4 \pm 0.5\%$) or saline alone ($HbA_{1c} = 9.5 \pm 1.0\%$) for 18 days (Saha et al 2006). These results indicate that VO(tpps) treatment provides glycaemic control not only in type 1 diabetic mice but also in type 2 diabetic mice. Serum UN concentration was lowered significantly in VO(tpps)-treated KKA$^y$ mice compared with that in saline- and VS-treated KKA$^y$ mice. GOT and GPT concentrations in VO(tpps)-treated KKA$^y$ mice were also significantly suppressed compared with those in saline-treated KKA$^y$ mice. Serum TCHO concentration in KKA$^y$ mice following treatment with VO(tpps) was lower as compared with that in KKA$^y$ mice treated with saline alone, indicating that cholesterol metabolism was improved by the treatment with VO(tpps). This supports the previous finding that vanadium compounds treat hypercholesterolaemia in KKA$^y$ mice (Adachi et al 2006). Serum TG concentration was lowered in VO(tpps)-treated KKA$^y$ mice as compared with that in saline-treated KKA$^y$ mice. On the other hand, serum TG concentration in VS-treated KKA$^y$ mice was not improved as compared with that in saline-treated KKA$^y$ mice. In contrast, concentrations of TG were unchanged in diabetic animals after treatment with sodium orthovanadate (Brichard et al 1990; Pugazhenthi et al 1991; Thompson et al 1999). However, it was reported that chronic oral administration of the bis(maltolato)oxovanadium(IV) complex, $VO(ma)_2$, reduced the plasma TG concentrations in Zucker diabetic fatty rats (Yuen et al 1999). Diabetes is associated with hyperlipidaemia. Therefore, the serum-TG-lowering effect of VO(tpps) is beneficial in the diabetic state. Serum FFA concentrations were almost the same in all groups of KKA$^y$ mice. It is noteworthy that both serum insulin and leptin concentrations in VO(tpps)-treated KKA$^y$ mice were reduced significantly compared with those in saline-treated KKA$^y$ mice, indicating

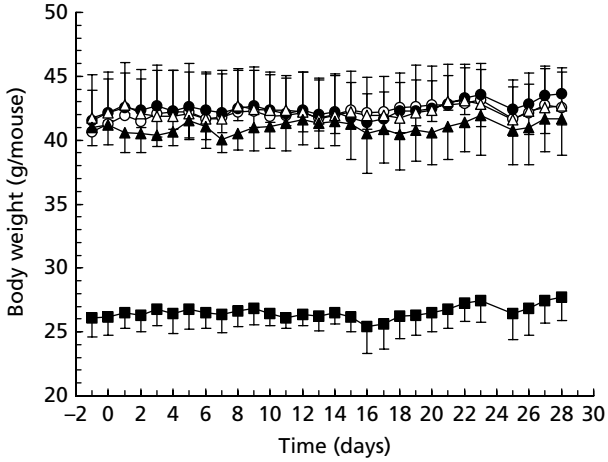


**Figure 4** Changes of body weight in saline-treated non-diabetic C57BL/6J mice (closed squares, n = 5) and type 2 diabetic KKA$^y$ mice after oral administration of saline (open circles, n = 5), VS (open triangles, n = 5), VO(tpps) (closed triangles, n = 7) and $H_2$tpps (closed circles, n = 6).

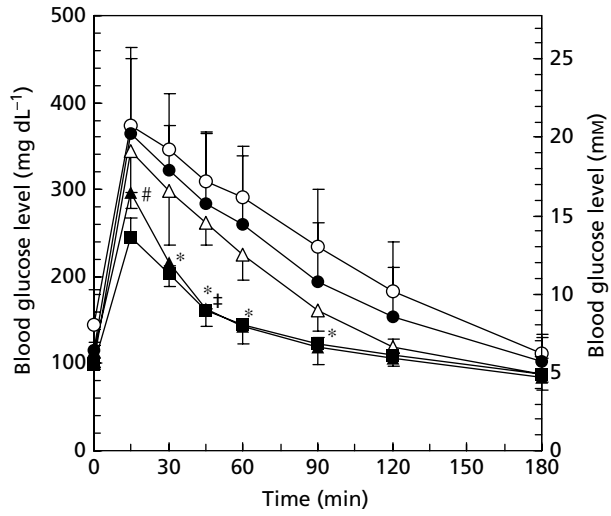


**Figure 5** Changes in blood glucose concentration during oral glucose tolerance tests (OGTT) for saline-treated non-diabetic CB57/6J mice (closed squares, n = 5) and type 2 diabetic KKA$^y$ mice treated with saline (open circles, n = 5), VS (open triangles, n = 5), VO(tpps), (closed triangles, n = 7) and $H_2$tpps (closed circles, n = 6). Following a 12-h fast, the OGTT was performed after VO(tpps) treatment. Glucose (1 g kg$^{-1}$) was administered by oral administration. *$P < 0.01$, #$P < 0.05$ vs saline-treated KKA$^y$ mice; ‡$P < 0.05$ vs VS-treated KKA$^y$ mice.

the improvement in both insulin and leptin resistance. These findings demonstrate that VO(tpps) has an anti-diabetic potency through not only its high blood glucose-lowering effect but also its ability to attenuate the metabolic syndromes involving lipid metabolism, insulin resistance and leptin resistance in type 2 diabetic KKA$^y$ mice. It has been reported that sodium orthovanadate and synthetic inhibitors of protein tyrosine phosphatase (PTPase) are effective in decreasing the plasma concentrations of glucose and insulin in *ob/ob* mice (Brichard et al 1990; Serdy et al 1995; Wrobel et al 1999). These results indicated that the diabetic state in *ob/ob* mice is associated with a reduction in insulin-induced protein tyrosine phosphorylation. The chronic oral administration of $VO(ma)_2$ significantly reduced the plasma insulin concentration in Zucker diabetic fatty rats (Yuen et al 1999). It was also demonstrated that oral $VO(ma)_2$ reduced the activity of protein tyrosine phosphatase 1B (PTP1B) in the skeletal muscle of fatty Zucker rats by 25% (Mohammad et al 2002). PTP1B is a specific tyrosine phosphatase that inhibits both leptin and insulin signal transductions (Zabolotny et al 2002, 2004).

In this study, a reduction in leptin concentration in KKA$^y$ mice treated with VO(tpps) was observed, which indicates the enhancement of leptin sensitivity in target organs by this complex. It may, therefore, be concluded that VO(tpps) regulates excessive food and water intake (Figure 3A, B), which in turn suppresses the progress of obesity (Figure 4). Moreover, the

**Table 2** $HbA_{1C}$ and serum parameters in saline-treated non-diabetic C57BL/6J and KKA$^y$ mice treated with saline alone, vanadium(IV) oxide sulfate (VS), VO(tpps) and $H_2$tpps in saline after daily oral administration for 28 days

| | **C57BL/6J mice** | **KKA$^y$ mice** | | | |
|---|---|---|---|---|---|
| | Saline | Saline | VS | VO(tpps) | $H_2$tpps |
| Dose (mg V/kg) | | | 5–10 | 5–10 | |
| Dose (mmol kg$^{-1}$) | | | (0.1–0.2) | (0.1–0.2) | (0.1–0.2) |
| N | 5 | 5 | 5 | 7 | 6 |
| $HbA_{1C}$ (%) | 3.6 ± 0.2 | 10.5 ± 1.3 | 9.1 ± 0.5 | 5.5 ± 1.2*† | 10.6 ± 0.5 |
| UN (mg dL$^{-1}$) | 25.9 ± 1.9 | 32.3 ± 3.9 | 28.3 ± 1.3 | 24.2 ± 2.9*‡ | 27.8 ± 3.4 |
| GOT (U L$^{-1}$) | 66 ± 6 | 67 ± 9 | 64 ± 9 | 56 ± 8# | 67 ± 13 |
| GPT (U L$^{-1}$) | 16 ± 2 | 22 ± 3 | 19 ± 4 | 15 ± 1* | 22 ± 8 |
| TG (mg dL$^{-1}$) | 85 ± 8 | 220 ± 69 | 220 ± 27 | 187 ± 8 | 232 ± 50 |
| TCHO (mg dL$^{-1}$) | 103 ± 13 | 154 ± 13 | 152 ± 29 | 129 ± 6* | 161 ± 15 |
| FFA (mEq L$^{-1}$) | 0.904 ± 0.101 | 1.725 ± 0.151 | 1.693 ± 0.061 | 1.725 ± 0.151 | 1.628 ± 0.347 |
| Insulin ($\mu$U mL$^{-1}$) | 7.7 ± 1.9 | 26.6 ± 8.7 | 22.4 ± 2.2 | 9.5 ± 3.8*† | 22.6 ± 5.6 |
| Leptin (ng mL$^{-1}$) | 3 ± 1 | 46 ± 9 | 45 ± 6 | 28 ± 9#‡ | 57 ± 12 |

*$P < 0.01$, #$P < 0.05$ vs saline-treated KKA$^y$ mice; †$P < 0.01$, ‡$P < 0.05$ vs VS-treated KKA$^y$ mice.

leptin concentration of KKA$^y$ mice treated with VO(tpps) is correlated with the insulin concentrations (Table 2). It has been suggested that both leptin and insulin may mutually modulate the secretion and production of each other (Kieffer & Habener 2000). Recently, we evaluated the mode of action for anti-diabetic activity of vanadium compounds, VS, bis(picolinato)oxovanadium(IV) complex, $VO(pa)_2$ and $VO(ma)_2$, in isolated rat adipocytes using inhibitors for glucose and fatty acid metabolism. Oxovanadium(IV) ion and its complexes have been revealed to act on at least four sites involving insulin-dependent signal transduction system, glucose transporter and phosphodiesterase (Kawabe et al 2006). Our study on the action mechanism responsible for the anti-diabetic and anti-metabolic syndrome activity of VO(tpps) is continued, and the results will be reported.

## Conclusion

VO(tpps) is an orally active oxovanadium(IV)–porphyrin complex with a $VO(N_4)$ coordination environment that is useful for treating not only type 1 diabetic STZ mice but also type 2 diabetic KKA$^y$ mice. In addition, VO(tpps) improves diabetes, obesity and metabolic syndromes by enhancing insulin sensitivity and leptin resistance in obesity-linked type 2 diabetic animals.

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