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Original Article

Development of new insulinomimetic zinc(II) picolinate complexes with a $Zn(N_2O_2)$ coordination mode: structure characterization, in vitro, and in vivo studies

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Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications nos. CCDC 156878 (**2**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

Abstract. Three zinc(II) complexes of picolinic acid and its derivatives with a $Zn(N_2O_2)$ coordination mode were prepared and evaluated for their insulinomimetic activities by in vitro and in vivo studies. By introducing an electron-donating methyl group into the picolinate ligand (pic), bis(6- or 3-methylpicolinato)zinc(II) complexes [$Zn(6\text{-mpa})_2$ or $Zn(3\text{-mpa})_2$, respectively] were prepared. The $Zn(6\text{-mpa})_2$ complex was crystallized as a water adduct $[Zn(6\text{-mpa})_2(H_2O)]\cdot H_2O$, in which two carboxylate oxygens and two pyridine nitrogens of 6-mpa and a water oxygen coordinate to a zinc(II) with a trigonal bipyramidal geometry. By in vitro evaluation of the inhibition of free fatty acid (FFA) release from isolated rat adipocytes in the presence of epinephrine, the insulinomimetic activities of $Zn(pic)_2$, $Zn(6\text{-mpa})_2$, and $Zn(3\text{-mpa})_2$ (IC_{50}=0.64±0.13, 0.31±0.05, and 0.40±0.07 mM, respectively) were found to be higher than those of $VOSO_4$ (IC_{50}=1.00 mM) and $ZnSO_4$ (IC_{50}=1.58±0.05 mM) in terms of IC_{50} value, the 50% inhibition concentrations for the FFA release from the adipocytes. Then, $Zn(6\text{-mpa})_2$, which exhibited the highest in vitro insulinomimetic activity among three complexes examined, was given at a dose of 3.0 mg (45.9 μmol) Zn/kg body weight to KK-A^y mice with type 2 diabetes mellitus by daily intraperitoneal injections for 14 days and it was found that the hereditary high blood glucose levels were lowered during the administration of the

complex. The improvement of diabetes mellitus was confirmed with the oral glucose tolerance test.

Keywords. Zinc(II) complex - Picolinic acid derivatives - Insulinomimetic activity - KK-A^{y} mice - Blood glucose normalizing effect

Abbreviations. *BUN*: blood urea nitrogen *FFA*: free fatty acids *GOT*: glutamic oxaloacetic transaminase *GPT*: glutamic pyruvic transaminase *KRB*: Krebs-Ringer bicarbonate *STZ*: streptozotocin *TCHO*: total cholesterol

Introduction

Some metal ions mimic the action of insulin in both in vitro and in vivo systems [*1*, *2*, *3*, *4*, *5*]. Among them, zinc(II) was found in 1980 to stimulate rat adipocytes lipogenesis similar to the action of insulin [*6*]. Then several research groups confirmed the insulinomimetic in vivo activity of zinc(II) [*7*, *8*, *9*]. Zinc(II), a known essential trace element in animals and humans, with a relatively low toxicity profile [*10*], has been shown to have a pronounced blood glucose normalizing effect [*11*]. Shisheva et al. [*11*] have reported that $ZnCl_2$ stimulated glucose uptake in rat adipocytes and reduced blood glucose concentrations as much as 50% when given orally to STZ rats (210 mg Zn/kg body wt). Furthermore, Chen et al. [*12*] have reported that the blood glucose level was normalized by the administration of 20 mM $ZnCl_2$ to *ob/ob* mice. Based on these results, it was concluded that zinc(II) has an insulinomimetic activity in vitro and in vivo [*11*, *12*].

However, insulinomimetic zinc(II) complexes with higher lipophilic characteristics than zinc(II) have not yet been reported so far. During the investigations to find insulinomimetic metal complexes, we found that bis(maltolato)- and bis(2-hydroxypyridine *N*-oxido)zinc(II) complexes [$Zn(mal)_2$ and $Zn(hpo)_2$, respectively] with a $Zn(O_4)$ coordination mode have higher insulinomimetic activity than free zinc(II) as estimated by in vitro experiments [*13*]. On the basis of these observations, we have aimed to develop more active zinc(II) complexes than $Zn(mal)_2$ and $Zn(hpo)_2$. Previously, picolinic acid as a chelating agent was reported to promote the absorption of zinc(II) into the body from the small intestine [*14*]. Therefore, we have chosen picolinic acid and its derivatives as ligands to zinc(II) and synthesized new complexes with a $Zn(N_2O_2)$ coordination mode.

This paper reports the structure-activity relationship by in vitro experiments in terms of the effect of the methyl group on the picolinic acid and the blood glucose lowering effects in KK-A^{y} mice which were given bis(6-methylpicolinato)zinc(II), $Zn(6\text{-mpa})_2$, with had the highest in vitro activity among the complexes examined.

Materials and methods

Materials and physical measurements

Zinc sulfate and acacia were purchased from Wako (Osaka, Japan). Picolinic acid and derivatives were obtained from Tokyo Kasei (Tokyo, Japan). D-(+)-Glucose was obtained from Nacalai Tesque (Kyoto, Japan). (±)-Epinephrine hydrochloride, collagenase, and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, Mo., USA). The purity of $ZnSO_4 \cdot 7H_2O$ was determined by chelatometry using the Cu-Pan [Cu-1-(2-pyridylazo-2-naphthol] complex (Dojindo, Kumamoto, Japan). All other reagents were of analytical or reagent grade and were used without purification. Elemental analyses of the complexes were carried out on a Perkin-Elmer 240C Elemental Analyzer. FT-IR spectra were recorded with KBr pellets on a Jasco FT/IR-420 spectrophotometer.

Preparation of zinc(II) complexes

Bis(picolinato)zinc(II), $Zn(pic)_2$ **1**, and bis(6-methylpicolinato)zinc(II), $Zn(6\text{-}mpa)_2$ **2**, were prepared according to the methods previously described [*15*, *16*]. Bis(3-methylpicolinato)zinc(II), $Zn(3\text{-}mpa)_2$ **3**, was prepared by addition of an aqueous solution of $ZnSO_4 \cdot 7H_2O$ (0.14 g , 0.5 mmol) to an aqueous solution of 3-methylpicolinic acid (0.14 g, 1.0 mmol) and $LiOH \cdot H_2O$ (0.04 g, 1.0 mmol), followed by stirring for 6 h at room temperature. The white precipitate obtained was washed with small amounts of water, methanol, and diethyl ether; yield: 0.14 g (63%). Compound **3** was characterized by its physicochemical properties. The physical parameters of **3** are as follows: anal. found: C, 46.83; N, 7.80; H, 3.91%; calcd for $C_{14}H_{12}N_2O_4Zn \cdot 1.2H_2O$: C, 46.80; N, 7.80; H, 4.04%. Mp >300 °C (dec.). IR (KBr disk): 1666 cm^{-1} for $\nu_{C=O}$.

X-ray data collection and structure determination

Colorless single crystals of **1** and **2** were obtained by recrystallization from a concentrated aqueous solution and methanol solution, respectively, and used for X-ray structure analysis. The data collections were performed with monochromated Mo Kα radiation for **1** and **2** on a Rigaku AFC7R diffractometer. All the calculations, including data reduction, were performed using a teXsan crystallographic software package (Molecular Structure Corporation). The structures of $\mathbf{1} \cdot 4H_2O$ and $\mathbf{2} \cdot 2H_2O$ were solved by direct methods (SIR92) and refined by the full-matrix least-squares procedure. The details of the crystallographic experiment and computation for the present complexes are listed in the Supplementary material.

Measurement of the partition coefficients

The partition coefficients of the zinc(II) complexes were determined by a conventional method in an *n*-octanol/saline system using a UV spectrometer (Hitachi U-3500L). The concentrations of the complexes in each phase were monitored at the characteristic wavelength of 270 nm due to the pyridine ring. The partition coefficients of the complexes were calculated by the equilibrium concentrations of the complexes in *n*-octanol and saline after shaking for 2 h at 37 °C.

Interaction of zinc(II) complexes with isolated rat adipocytes treated with epinephrine

Isolated male rat adipocytes (1.0×10^6 cells/mL) prepared as described [*17*, *18*, *19*, *20*] were

preincubated at 37 °C for 30 min with various concentrations (10^{-4}-10^{-3} M) of the zinc(II) complexes in KRB buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$; pH 7.4) containing 2% BSA but without glucose. A 10^{-4} M epinephrine solution was then added to the reaction mixtures and the resulting solutions were incubated at 37 °C for 180 min. The reactions were stopped by soaking in ice water and the mixtures were centrifuged at 3000 rpm for 10 min. For the outer solution of the cells, free fatty acid (FFA) levels were determined with an FFA kit (Wako, Osaka, Japan).

In vivo evaluation of 2 on the lowering of blood glucose levels in KK-A^y mice

In the present study, we used KK-A^y mice as a congeneric strain, in which the A^y allele at the agouti locus (initially from C57B/6J-A^y) had been transferred to the inbred KK strain by repetitive back crossing. The introduction of the A^y allele causes overt diabetes and massive obesity hereditarily. The KK-A^y mice (8 weeks old; CREA Japan, Tokyo) with type 2 diabetes mellitus, which were housed in an air-conditioned room at a temperature of 23±1 °C and 60±10% humidity with lights on from 8:00 a.m. to 8:00 p.m., received daily intraperitoneal (i.p.) injections (five mice in a group) of **2** dissolved in a 5% acacia vehicle at about 10 a.m. after the determination of their blood glucose levels, for 2 weeks. Acacia was used to increase the solubility of the complex in saline. The blood sample for the analysis of the glucose level was obtained from the tail vein of each mouse and measured with a Glucocard (Arkray, Kyoto, Japan). The body weights of the KK-A^y mice, which were allowed free access to solid food (CREA Japan; zinc content was 6.25 mg/100 g) and tap water, were measured daily during the administration of **2**. Intakes of solid food and drinking water in each mouse were checked daily throughout the experiments. A dose of 25.1 mg of **2**/kg body weight corresponds to 3.0 mg (45.9 µmol) Zn/kg body weight. The blood samples for the analyses of blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and total cholesterol (TCHO) were withdrawn from the cavernous sinus with a capillary under anesthesia with ether. The serum concentrations of BUN, GOT, GPT, and TCHO were determined by Fuji Dry Chem (Fuji Medical, Tokyo, Japan).

The animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University (KPU) and was performed according to the Guideline for Animal Experimentation of KPU.

Determination of HbA1C

Hemoglobin A_{1C} was measured by an immunoassay method (DCA2000 System, Bayer-Sankyo, Tokyo, Japan).

Oral glucose tolerance test

After the administration of complex **2** for 14 days, the mice were fasted for 12 h and glucose at a dose of 1 g/kg body weight was given orally. Blood samples were obtained from a tail vein at 0, 30, 60, 90, and 120 min after the glucose administration, and the blood glucose levels were measured with the Glucocard.

Results and discussion

The zinc(II) complex *trans*-$[Zn(pic)_2(H_2O)_2]\cdot 2H_2O$ **1** was prepared according to the method previously described [*15*, *16*]. A colorless single crystal of **1** suitable for X-ray structure analysis was obtained from an aqueous solution. Although X-ray crystal data for **1** have been reported, we used our own data because of their improved quality. The coordination geometry around the zinc(II) in **1** was confirmed to be an octahedral structure, as reported by Yunuskhodzhaev et al. [*16*]. Another zinc(II) complex, **2**, was prepared and a colorless single crystal suitable for X-ray structure analysis was obtained from a methanol solution. The coordination geometry around the zinc(II) in **2** was evaluated to be a distorted trigonal bipyramidal structure (Fig. 1). The zinc(II) lies in an equatorial plane (O1, O1′, and O3) with two apical atoms (N1 and N1′).

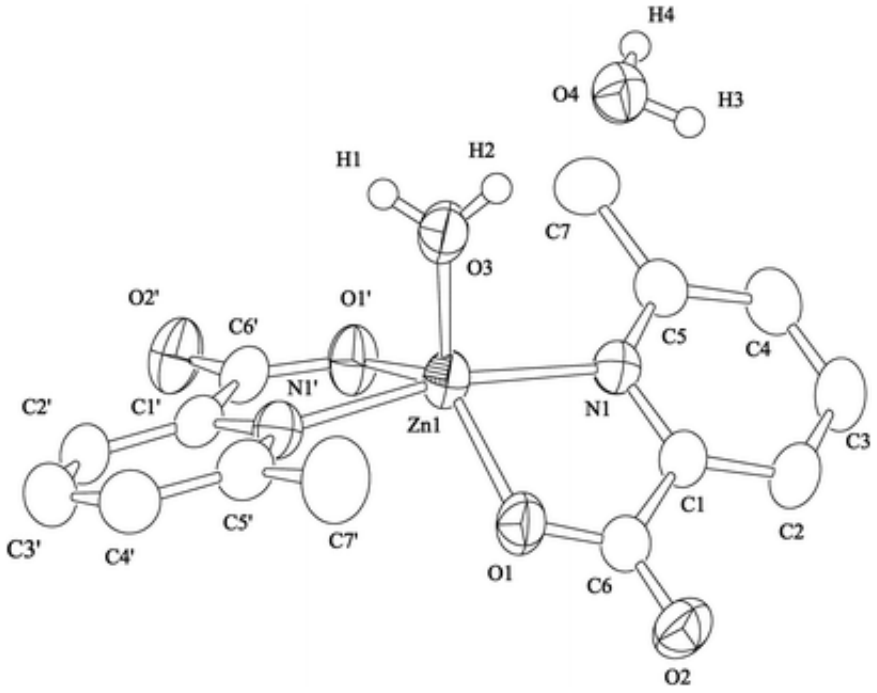

Fig. 1.

The partition coefficients were determined by a conventional method in an *n*-octanol/saline system. The partition coefficients of **1**, **2**, and **3** are shown in Table 1, in which introduction of a methyl group is indicated to enhance the lipophilicity.

Table 1. The partition coefficients of the zinc(II) complexes **1**, **2**, and **3**

Complex	Partition coefficient[a]
1	0.018±0.01
2	0.075±0.02[b]
3	0.070±0.01[b]

[a] Data are expressed as the mean±SD for three experiments

[b] Significance at $P<0.01$ versus **1**

The in vitro insulinomimetic activities of the three complexes were evaluated by the inhibition of the FFA release from isolated rat adipocytes in the presence of epinephrine [*17*]. The in vitro evaluation method used has been proven to be very effective and convenient in finding vanadyl complexes with antidiabetic activity [*18*, *19*, *20*]. The apparent insulinomimetic activity of the complexes was estimated by the IC_{50} value, which expresses the 50% inhibitory concentration of each complex. As shown in Fig. 2, dose-dependent inhibitory effects of FFA release were observed, and the IC_{50} values were calculated. Zinc(II) complexes at a concentration of about 5×10^{-4} M inhibited FFA release from the epinephrine-stimulated rat adipocytes stronger than $VOSO_4$ or $ZnSO_4$. From these results, the apparent IC_{50} values were estimated to be 1.00, 1.58±0.05, 0.64±0.13, 0.31±0.05, and

0.40±0.07 mM for $VOSO_4$, $ZnSO_4$, **1**, **2**, and **3**, respectively (Table 2). The introduction of a methyl group at the 6- or 3-position of the picolinic acid enhanced insulinomimetic activity more than that of **1**, indicating that the lipophilicity of a complex is very important in enhancing the activity of the complex, as observed also in the activity of vanadyl complexes [*19*]. In addition, ligand such as pic and 6-mpa alone gave no inhibitory effects on the FFA release. Shisheva et al. [*11*] reported that a [*N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine]zinc(II) complex with a $Zn(N_4)$ coordination mode does not have any insulinomimetic activity. We also confirmed this by an in vitro experiment, namely a (triethylenetetraamine)zinc(II) complex **4** with a $Zn(N_4)$ coordination mode exhibited no insulinomimetic activity (Fig. 2 and Table 2). Thus, the complexes with a $Zn(N_2O_2)$ coordination mode have been found to have stronger insulinomimetic activities than free vanadyl [VO(II)], free zinc(II), or zinc(II) complexes with a $Zn(N_4)$ coordination mode.

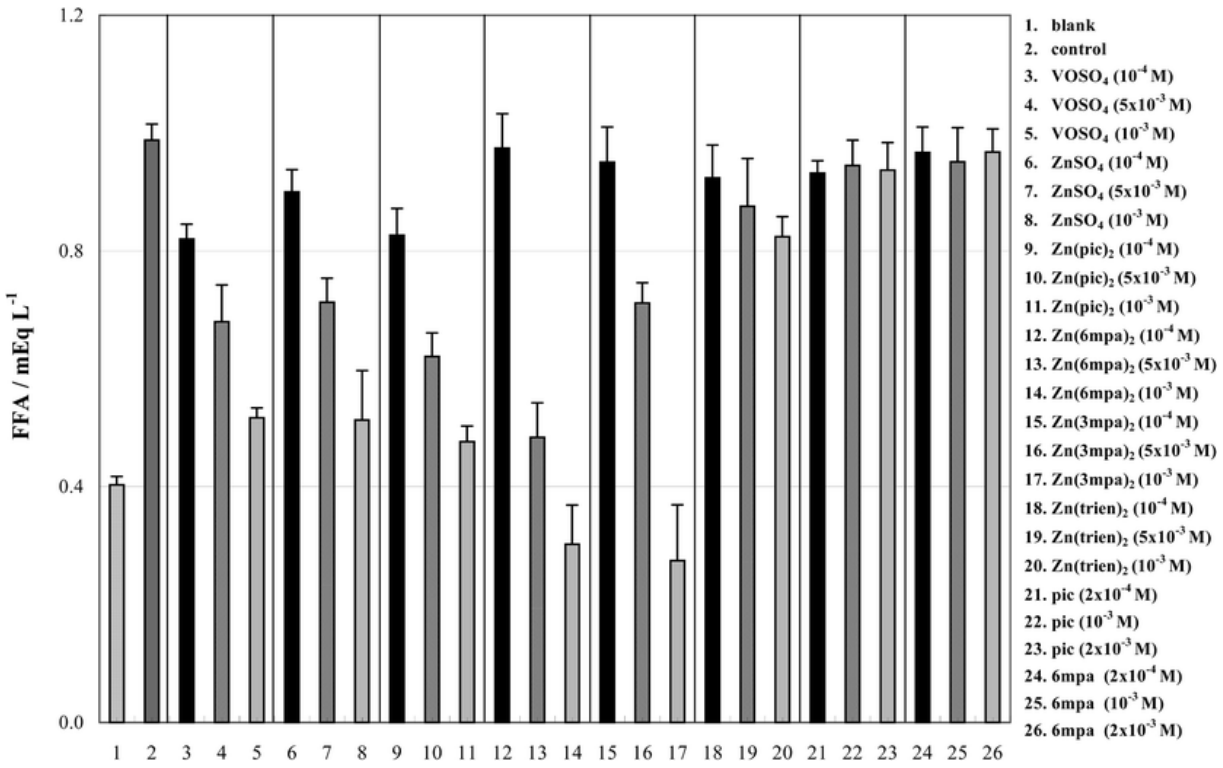

Fig. 2.

Table 2. Estimated IC_{50} values for the free fatty acid release from rat adipocytes

Complex	IC_{50} (mM)[a]
$VOSO_4$	1.00
$ZnSO_4$	1.58±0.05
1	0.64±0.13[b]
2	0.31±0.05[c]
3	0.40±0.07[c]
4	None
Picolinic acid	None
6-Methylpicolinic acid	None

[a] Data are expressed as the mean±SD for three experiments

[b] Significance at $P<0.05$ versus $VOSO_4$

[c] Significance at $P<0.01$ versus $VOSO_4$

Based on the in vitro insulinomimetic activities of the zinc(II) complexes, we examined the in vivo insulinomimetic activity in terms of the blood glucose normalization effect by daily i.p. injections of **2**, which exhibited the highest activity in the in vitro evaluation. At first, we tried to use the streptozotocin-induced diabetic rats (STZ rats). However, the safety margin of the dose of zinc(II) was very narrow because the absorption of zinc(II) in STZ rats is higher than in normal rats [*21*]. Therefore, it was very difficult to determine the dose of the zinc(II) complexes. For this reason, we used KK-A^y mice with a hereditary type 2 diabetes mellitus, in which the dosage was not so difficult to determine.

The blood glucose levels and the body weight changes of KK-A^y mice given **2** are shown in Fig. 3. When **2** was injected at a dose of 3.0 mg (45.9 μmol) Zn/kg of body weight for the first 2 days, the blood glucose levels were lowered to about 13.9 mM (250 mg/dL), and it was maintained for the following 2 weeks by daily administration of the same dose (Fig. 3, left). During the treatment of **2** for 2 weeks, the body weight of the KK-A^y mice was unchanged for the first 6 days but then increased slightly from 37.6±1.4 to 40.1±1.4 g (Fig. 3, right).

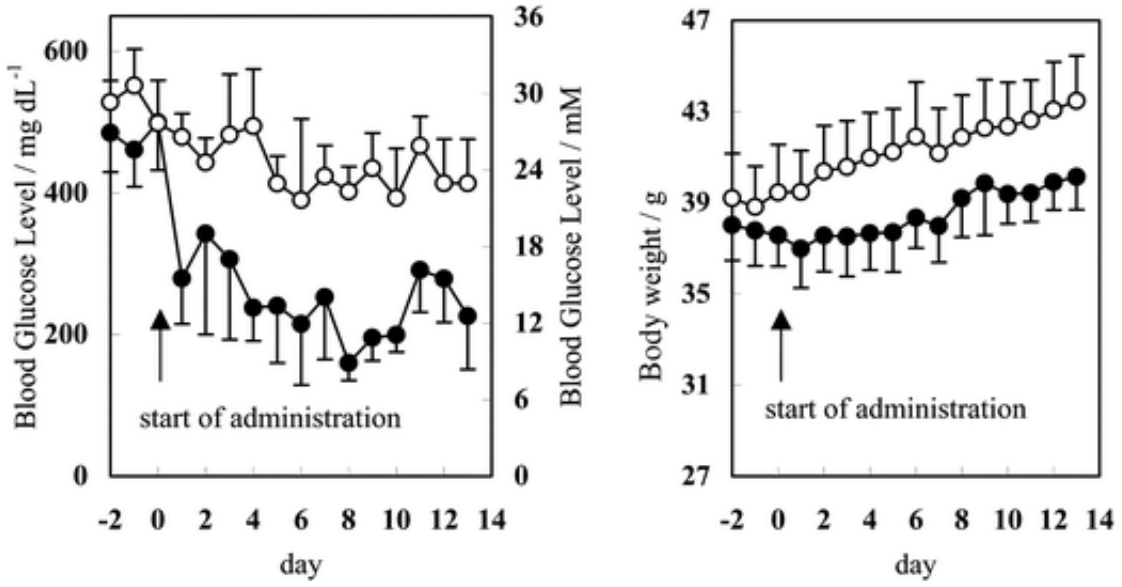

Fig. 3.

The BUN, GOT, GPT, and TCHO levels of the KK-A^y mice given 5% acacia vehicle alone (untreated KK-A^y mice) and KK-A^y mice treated with **2** for 2 weeks are summarized in Table 3. The BUN level, which indicates the degree of renal disturbance, did not change compared with those of the untreated KK-A^y mice. The GOT and GPT levels, which show the degree of hepatic disturbance, were also identical to those of the untreated KK-A^y mice. The TCHO levels were lowered by the treatment with **2**, suggesting that the cholesterol metabolism was improved by the administration of the complex. From these results, it was suggested that **2** has a low toxicity in hepatic and renal functions, and improves cholesterol metabolism.

Table 3. Serum parameters of KK-A^y mice receiving daily i.p. injections of **2**[a]

Treatment	BUN (mg/dL)	GOT (U/L)	GPT (U/L)	TCHO (mg/dL)
KK-A^y mice	32.9±2.4	61±15	30±9	176±48
KK-A^y mice+**2**	27.3±9.4	59±14	36±17	127±10[b]

[a] Data are expressed as the mean±SD for five mice of the untreated KK-A^y mice and KK-A^y mice receiving daily i.p. injection of **2** for 2 weeks. Experimental conditions are as for Fig. 3

[b] Significance at $P<0.01$ versus KK-A^y mice

Furthermore, we examined the changes of HbA$_1$c in the untreated KK-A^y mice and KK-A^y mice treated with **2** (Table 4). Recently, the measurement of HbA$_1$c has been used as an index of glycemic control in diabetic patients [*22*, *23*]. In the untreated KK-A^y mice, the HbA$_1$c level increased from 7.6±0.5% at 8 weeks to 8.3±0.3% at 10 weeks. However, the HbA$_1$c levels of KK-A^y mice treated with **2** gave a tendency to slightly decrease from 6.8±0.4% at 8 weeks to 6.3±0.6% at 10 weeks. These results indicated that the diabetic state of KK-A^y mice treated with **2** was improved from that of the untreated KK-A^y mice in terms of glucose and HbA$_1$c levels. Previously, it was reported that the administration of zinc(II) complexes for type 1 diabetes patients increased the HbA$_{1C}$ level [*24*];

however, the HbA_1c level of the mice with type 2 diabetes mellitus and treated with complex **2** was decreased.

Table 4. HbA_{1C} levels of KK-A^y mice receiving daily i.p. injections of **2**

Treatment	Day	HbA_{1C} level[a]
KK-A^y mice	0	7.6±0.5[b]
	14	8.3±0.3[b]
KK-A^y mice+**2**	0	6.8±0.4
	14	6.3±0.6

[a] Data are expressed as the mean±SD for four mice of untreated KK-A^y mice and KK-A^y mice received daily i.p. injection of **2** for 2 weeks. Experimental conditions are as for Fig. 3

[b] Significance at $P<0.05$ versus HbA_{1C} level at 0 day

Because the blood glucose levels of KK-A^y mice treated with **2** for 2 weeks were lowered to below 250 mg/dL (13.9 mM), the animals received the oral glucose tolerance test. As shown in Fig. 4, the blood glucose levels of the untreated KK-A^y mice were elevated to the maximum [270 mg/dL (15.0 mM)] at 30 min after the glucose administration, and then it gradually decreased. In contrast, the blood glucose levels of KK-A^y mice treated with **2** were also slightly elevated for the first 30 min, but they were obviously lowered when compared with those of the untreated KK-A^y mice. These results revealed that the treatment of **2** mitigated the diabetes in KK-A^y mice by improving glucose tolerance.

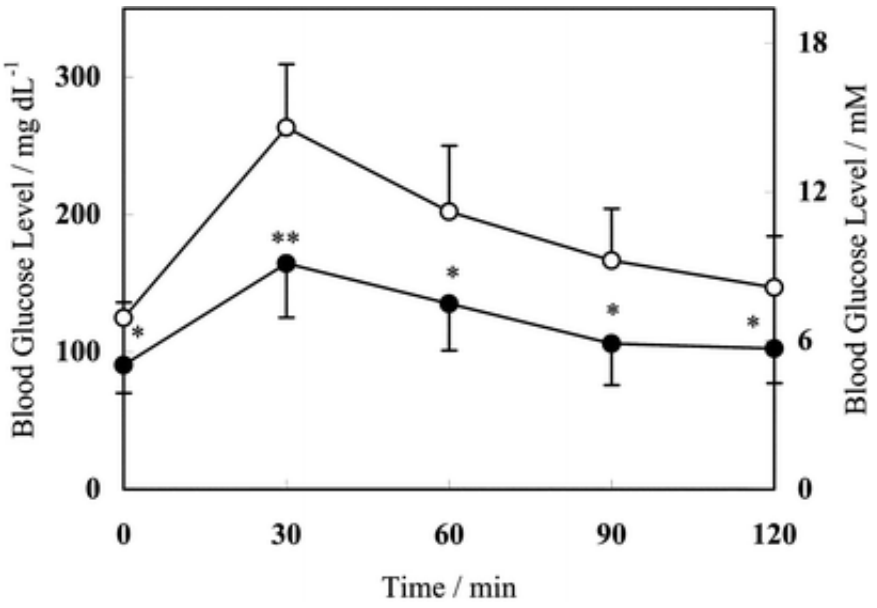

Fig. 4.

Previously, we reported that the vanadyl analogue bis(6-methylpicolinato)oxovanadium(IV) [$VO(6\text{-mpa})_2$] of $Zn(6\text{-mpa})_2$ exhibits a high insulinomimetic in vivo activity for not only type 1 diabetic STZ rats [*18*] but also type 2 diabetic KK-A^y mice [*25*]. Although the in vitro IC_{50} values of $Zn(6\text{-mpa})_2$ and $VO(6\text{-mpa})_2$ [*26*] were in the same range, 0.31±0.05 mM and 0.49±0.08 mM, respectively, the in vivo insulinomimetic activity in KK-A^y mice given i.p. injections of $Zn(6\text{-mpa})_2$ at a dose of 3.0 mg (45.9 μmol) Zn/kg body weight was found to be higher and more stable than that given $VO(6\text{-mpa})_2$ at a similar dose of 2.5 mg (49 μmol) V/kg body weight [*25*].

In conclusion, we propose here a new zinc(II) complex, $Zn(6\text{-mpa})_2$, with a $Zn(N_2O_2)$ coordination mode as a potent insulinomimetic agent against type 2 diabetes mellitus. The present study suggests that the introduction of a methyl group into the picolinate ligand is a useful method to develop more active insulinomimetic complexes than the leading $Zn(pic)_2$ complex, as evaluated by in vitro and in vivo experiments. The detailed mechanism of the blood glucose lowering effect and a toxicological study for the clinical use of the complex are now under way.

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References

1.Thompson KH, McNeill JH, Orvig C (1999) Chem Rev 99:2561-2571

2.Clifford JB (1999) Diabetes Rev 7:94-113

3.Schwarz K, Mertz W (1959) Arch Biochem Biophys 85:292-295

4.Rubenstein AH, Levin NW, Elliott GA (1962) Nature 194:188-189

5.Cohen N, Halberstam M, Shlimovich P, Chang CJ, Shamoon H, Ressetti L (1995) J Clin Invest 95:2501-2509

6.Coulston L, Dandona P (1980) Diabetes 29:665-667

7.May JM, Contoreggi CS (1982) J Biol Chem 257:4362-4368

8.Ezaki O (1989) J Biol Chem 264:16118-16122

9.Kasuga M, Karrlson FA, Kahn CR (1982) Science 215:185-187

10.Underwood EJ (1977) In: Trace elements in human and animal nutrition, 4th edn. Academic Press, New York, pp 230-245

11.Shisheva A, Gefel D, Schechter Y (1992) Diabetes 41:982-988

12.Chen MD, Liou SJ, Lin PY, Yang VC, Alexander PS, Lin WH (1998) Biol Trace Elem Res 61:303-311

13.Yoshikawa Y, Ueda E, Kawabe K, Miyake H, Sakurai H, Kojima Y (2000) Chem Lett 874-875

14.Barrie SA, Wright JV, Pizzorno JE, Kutter E, Barron PC (1987) Agents Actions 21:223-228

15.Takenaka A, Utsumi B, Ishihara H, Furusaki A, Hitta I (1970) J Chem Soc Jpn 91:921-927

16.Yunuskhodzhaev AH, Mukarramova UA, Karimov Z (1998) Kristallografiya 43:42-44

17.Nakai M, Watanabe H, Fujisawa C, Satoh T, Takada J, Matsushita R, Sakurai H (1995) Biol Pharm Bull 18:719-725

18.Fujimoto S, Fujii K, Yasui H, Matsusita R, Takada J, Sakurai H (1997) J Clin Biochem Nutr 23:113-129

19.Kawabe K, Tadokoro M, Ichimura A, Kojima Y, Takino T, Sakurai H (1999) J Am Chem Soc 121:7937-7938

20.Sakurai H, Tsuji A (1998) In: Nriagu JO (ed) Vanadium in the environment. Part 2: health effects. Wiley, New York, pp 297-315

21.Neal EC, Mark LF (1983) Am J Physiol 244:E122-E128

22.Koenig RJ, Peterson CM, Kilo C, Cerami A, Williamson JR (1976) Diabetes 25:230-232

23.Nathan DM, Singer DE, Hurxthal K, Goodson JD (1984) New Engl J Med 310:341-346

24.Cunningham JJ, Aizhong F, Mearkle PL, Brown RG (1994) Metabolism 43:1558-1562

25.Fujisawa Y, Sakurai H (1999) Chem Pharm Bull 47:1668-1770

26.Takino T, Yasui H, Yoshitake Y, Matsushita R, Takada J, Sakurai H (2001) JBIC 6:133-142