A New Anti-diabetic Zn(II)–Hinokitiol (β-Thujaplicin) Complex with $Zn(O_4)$ Coordination Mode

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Insulin-mimetic and anti-diabetic activities of bis(hinokitiolato)Zn(II) complex, $Zn(hkt)_2$, with $Zn(O_4)$ coordination mode were evaluated. This complex was found to have the highest insulinomimetic activity among the Zn(II) complexes reported previously. In addition, the complex improved hyperglycemia, glucose tolerance, and insulin resistance.

In 2002, the number of patients suffering from diabetes mellitus (DM) globally reached 177 million.[1] DM is defined as a life-style related disease that develops chronic high blood glucose level resulting from the disorders in glucose, protein, and lipid metabolisms due to absolute or relative insulin deficiency; insulin is secreted by the beta cells of the islets of Langerhans in the pancreas.[2] According to WHO standards, DM can be divided into 2 types, type 1 DM and type 2 DM.[3] Type 2 DM is caused by lowering of insulin sensitivity that is triggered by eating habits, lack of exercise, obesity, and spiritual stress. This disease is treated by controlled diets, physical exercise, and oral medicines. Several types of synthetic therapeutics have been developed to treat type 2 DM, and these are being used clinically, however, severe side effects caused by their long-term use. Therefore, in the 21st century, development of a new class of pharmaceuticals to treat DM is required.[4,5] For this purpose, several metals and their complexes were examined and were discovered to exhibit anti-diabetic effects.[6–8] Our objective is to find a leading compound that exhibits strong insulin-mimetic activity and to study the structure–activity relationship of various compounds to discover more effective metal complexes. Previously, we have reported many types of vanadyl (VO(II)) and Zn(II) complexes.[9–13] In particular, we proposed that anti-diabetic Zn(II) complexes with the $Zn(O_4)$ coordination mode; Zn(II) complexes with maltol (mal) or allixin (alx), a component found in garlic, exhibited significant blood glucose lowering effects in KK-A^y mice with type 2 DM.[9,10,13] Based on these findings, we focused on the bis(hinokitiolato)Zn(II) complex, ($Zn(hkt)_2$),[14] with the same $Zn(O_4)$ coordination mode. Hinokitiol (β-Thujaplicin), which was found from *Chamaecyparis taiwanensis* by Nozoe in 1936, and the chemical structure was determined in 1944, is a natural compound with a wide variety of physiological functions that include anti-microbial, anti-tumor, reactive oxygen scavenging, and food preservation activities.[15–18] In this study, we discovered a new function of hinokitiol for antidiabetic activity when it was complexed with the Zn(II).

$Zn(hkt)_2$ was prepared as reported,[19] and the structure of $Zn(hkt)_2$ was estimated by elemental analyses, IR, and mass spectra.[14] The results suggest that the structure of the $Zn(hkt)_2$ complex contained a $Zn(O_4)$ coordination mode at the binding ratio of Zn(II):ligand = 1:2 (Figure 1).

The in vitro insulin-mimetic activity of Zn(II) compounds was determined by both FFA release inhibitory and glucose uptake enhancing abilities in isolated rat adipocytes treated with epinephrine, as previously reported.[20,21] Using both these assays, $Zn(hkt)_2$ was found to have remarkably higher activity than those of ionic $ZnSO_4$ and other Zn(II) complexes with the $Zn(O_4)$ coordination mode (Table 1). We have reported that the lipophilicity of the ligand in the Zn(II) complex is an important factor in developing an insulinomimetic complex because Zn(II) complexes act on several action points within the cells,[13] as named "ensemble mechanism."[22] In fact, hkt related compounds have high lipophilicities when they complex with metal ions.[23] Thus, the high insulinomimetic activity of $Zn(hkt)_2$ is due to its high lipophilicity. In addition, the ligand such as hinokitiol exhibited no inhibitory effect on the FFA release.

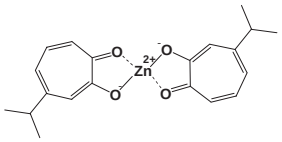

Figure 1. Estimated structure of the $Zn(hkt)_2$ complex.

KK-A^y mice, which are widely used as an excellent type 2 diabetic model, received daily intraperitoneal (*i.p.*) injections of $Zn(hkt)_2$ for 14 days, and their blood glucose levels and serum parameters were monitored.[24] As shown in Figure 2a, $Zn(hkt)_2$ exhibited a remarkable normoglycemic effect after 2–3 days of treatment, and the effect was maintained during the administration period of 14 days at an average dose of 2 mg Zn/kg body weight. After the treatment, the oral glucose tolerance test (OGTT) was performed in order to examine whether or not $Zn(hkt)_2$ improved glucose tolerance in KK-A^y mice. The elevated blood glucose level in KK-A^y mice was significantly suppressed by $Zn(hkt)_2$ administration (Figure 2b). The area under the curves (AUC) for the blood glucose levels were estimated to be 485 ± 66 and 370 ± 53 (mg/dL h) for the untreated KK-A^y mice, and $Zn(hkt)_2$ treated KK-A^y mice, respectively. No alterations of GOT, GPT, and UN levels, which are indicators for the development hepatotoxicity and nephrotoxicity to estimate the toxicity of $Zn(hkt)_2$ complex. Similarly no altera-

Table 1. Inhibitory effect of FFA release (IC_{50}) and effect of glucose uptake (EC_{50}) of Zn(II) complexes in adipocytes

Compound	Coordination mode	IC_{50} value (μM)	EC_{50} value (μM)
$ZnSO_4$	Ionic	413 ± 10	249 ± 18
$Zn(mal)_2$[13]	O_4	301 ± 10	174 ± 11
$Zn(alx)_2$[13]	O_4	151 ± 11	104 ± 4
$Zn(hkt)_2$	O_4	74 ± 31	45 ± 1
Hinokitiol		none	none

Table 2. HbA_{1c} and the serum parameters in KK-A^y mice treated with $Zn(hkt)_2$

	GOT (U/L)	GPT (U/L)	TG (mg/dL)	TCHO (mg/dL)	UN (mg/dL)	HbA_{1c} (%)	Insulin (μU/mL)	Leptin (ng/mL)	Adiponectin (ng/mL)
Control	116 ± 53	33 ± 16	102 ± 23	108 ± 10	32 ± 8	7.3 ± 1.1	11 ± 5	17 ± 6	32 ± 4
$Zn(hkt)_2$	146 ± 31	32 ± 8	120 ± 22	115 ± 32	25 ± 4	5.3 ± 0.4[a]	2.4 ± 4.4[b]	8 ± 4[a]	53 ± 8[a]

[a] $p < 0.05$ vs Control. [b] $p < 0.01$ vs Control.

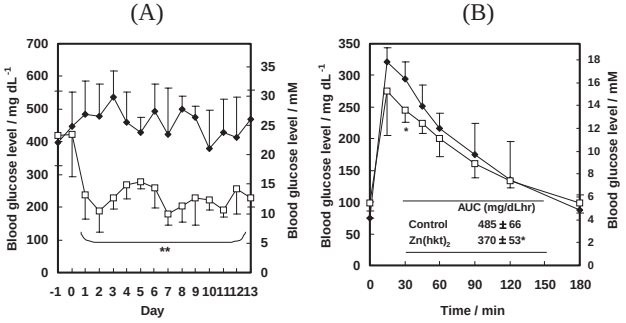

Figure 2. (A) Changes in the blood glucose levels in KK-A^y mice treated with 1% CMC (Control: ◆) and $Zn(hkt)_2$ (□). (B) Oral glucose tolerance test for control KK-A^y mice (◆) and KK-A^y mice receiving daily *i.p.* injections of $Zn(hkt)_2$ (□). Each point is expressed as the mean ±SD for five mice. Significance: $^*p < 0.05$ vs Control, $^{**}p < 0.01$ vs Control.

tions were observed in the TCHO or TG levels, which are indicators of fat metabolism (Table 2). KK-A^y mice are known to exhibit the diabetic state by developing both hyperinsulinemia and hyperleptinemia as a result of insulin resistance.[10,11,13] On $Zn(hkt)_2$ administration, these two parameters were significantly improved (Table 2). Leptin is an adipocyte hormone that is involved in the regulation of energy homeostasis[25] and obesity. Reduction of hyperinsulinemia and hyperleptinemia strongly indicated that $Zn(hkt)_2$ improved insulin resistance in all organs of KK-A^y mice. In addition, we have first discovered that adiponectin, an antidiabetic hormone, that decreases in type 2 DM with insulin resistance, increased on $Zn(hkt)_2$ administration (Table 2).

Based on these results, we conclude that the $Zn(hkt)_2$ complex has a remarkably strong anti-diabetic effect that acts by improving insulin and leptin resistances and subsequently increasing the adiponectin levels. We propose here that the $Zn(hkt)_2$ complex with $Zn(O_4)$ coordination mode is an extremely effective compound belonging to a new class of agents that can be used to treat DM.

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14 $Zn(hkt)_2$ complex: The complex was prepared in methanol by mixing $Zn(CH_3COO)_2$ and hinokitiol at a 1:2 molar ratio, and the solution was stirred for 2 h at room temperature. The resultant pale yellow precipitate was collected by vacuum filtration, washed with several times with pure methanol and was dried overnight in vacuo. Anal. Found: C, 59.97; H, 6.36%. Calcd for $C_{20}H_{22}O_4Zn \cdot 0.5H_2O$: C, 59.94; H, 5.78%. IR (KBr disk); 1591 (complex), 1608 (ligand) cm^{-1} for $\nu_{C=O}$. ESI MS; m/z 391 $[M + H]^+$.
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24 In vivo experiments; On *i.p.* injections of $Zn(hkt)_2$ suspended in 1% carboxymethyl cellulose, the doses were adjusted to 1–3 mg (15.3–45.9 μmol) of the Zn/kg body weight according to the blood glucose level observed. The body weight of the untreated KK-A^y mice and $Zn(hkt)_2$ treated mice increased from 38.3 ± 2.1 and 37.8 ± 3.0 to 40.6 ± 2.9 and 38.8 ± 2.5, respectively. After the treatment for 14 days, some serum parameters such as urea nitrogen (UN), GOT, GPT, triglycerides (TG), total cholesterol (TCHO), hemoglobin A_{1c} (HbA_{1c}), insulin, leptin, and adiponectin levels were measured, an OGTT was performed according to a previously method.[10,13]
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