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Oral administration of a zinc complex improves type 2 diabetes and metabolic syndromes

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Abstract

Previously, we reported that intraperitoneal injections of the Zn(II) complex ($Zn(alx)_2$) with allixin, which is isolated from dry garlic, with a $Zn(O_4)$ coordination environment, exhibited high anti-diabetic effects in obesity-linked type 2 diabetic KKA^y mice. However, this complex exhibited low activity when administered orally. To improve the effect of $Zn(alx)_2$, we prepared a novel Zn(II) complex with the allixin-derivative bis(1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4- thionato)Zn(II), abbreviated as Zn(II)–thioallixin-*N*-methyl ($Zn(tanm)_2$), having a $Zn(S_2O_2)$ coordination environment; this complex has extremely high *in vitro* insulin-like activity. Because Zn was extensively absorbed from the gastrointestinal tract when $Zn(tanm)_2$ was orally administered, its anti-diabetic effects were examined in KKA^y mice. Daily oral administrations of $Zn(tanm)_2$ for 4 weeks in KKA^y mice significantly improved hyperglycemia, glucose intolerance, insulin resistance, hyperleptinemia, obesity, and hypertension. Interestingly, $Zn(tanm)_2$ increased depressed plasma adiponectin levels in the mice. Here, we propose that $Zn(tanm)_2$ will be an orally active therapeutic for obesity-linked type 2 diabetes and metabolic syndromes.

Keywords: Zn(II) complex; Obesity-linked type 2 diabetes; Metabolic syndromes; Adiponectin; Leptin resistance; Insulin resistance; Hypertension

The worldwide prevalence of type 2 diabetes, which accounts for 90–95% of all diabetes, is continuing to grow annually by 6%, and it is expected to reach a total of 200–300 million cases in 2010 [1]. Several types of therapeutics for type 2 diabetes have been developed worldwide; however, they have limited efficacy and tolerability and occasionally cause severe side effects [2]. Therefore, approaches based on novel concepts are needed.

Zinc (Zn) is an essential trace element that is found in the entire body, and a wide spectrum of proteins, metalloenzymes, and transcription factors contain this metal ion. Zn(II) salts with low toxic profiles [3] have been shown to have anti-diabetic effects in diabetic experimental mice [4–6]. However, few studies on Zn(II) complexes have been reported. Previously, we proposed that the complexation of Zn(II) is advantageous as it enhances anti-diabetic activity, increases gastrointestinal absorption, and reduces Zn toxicity (as judged by its LD_{50} value) [7,8]. Encouraged by these findings, we have developed several types of Zn(II) complexes with various coordination environments to treat diabetes in experimental animals [7]. The anti-diabetic effects of the Zn(II) complexes have been evaluated in KKA^y mice because they are an excellent model that closely resembles type 2 diabetes in humans, including the expression of several disorders such as severe hyperglycemia, insulin

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resistance, obesity, hyperlipidemia, and hypertension in a single individual [9,10].

A Zn(II) complex with maltol ($Zn(ma)_2$) that exhibits high anti-diabetic activity by intraperitoneal (i. p.) injections in KKA^y mice was discovered in 2001 [11,12]. Hence, we studied the structure–activity relationships of its related complexes and have proposed a Zn(II) complex ($Zn(alx)_2$) with a $Zn(O_4)$ coordination environment with allixin (3-hydroxy-5-methoxy-6-methyl-2-pentyl-4*H*-pyrane-4-one) isolated from dry garlic [13]. However, oral administration of $Zn(alx)_2$ did not induce anti-diabetic effects in KKA^y mice, although the elevated HbA_{1c} levels were slightly lowered [14]. During our study on $Zn(alx)_2$-related complexes to discover more active complexes, we recently found a novel Zn(II) complex with thioallixin-*N*-methyl (1,6-dimethyl-3-hydroxy-5-methoxy-2-pentyl-1,4-dihydropyridine-4-thione), abbreviated as $Zn(tanm)_2$; this complex showed extremely high insulin-like activity in isolated rat adipocytes [15].

The aim of the present study was to test whether or not daily oral administrations of $Zn(tanm)_2$ improved diabetes and other metabolic abnormalities in obesity-linked type 2 diabetic KKA^y mice. On the basis of the results, we propose $Zn(tanm)_2$ with the $Zn(S_2O_2)$ coordination environment as a potent candidate for future clinical trials for the treatment of both hyperglycemia and metabolic syndromes that occur in obesity-linked type 2 diabetes.

Materials and methods

Chemicals. $Zn(alx)_2$ and $Zn(tanm)_2$ were prepared according to the methods described previously [13–15]. Allixin and thioallixin-*N*-methyl were obtained from Wakunaga Pharmaceutical Co. (Hiroshima, Japan). All other reagents used were of the highest purity commercially available.

Animals. Male C57BL/6J and type 2 diabetic KKA^y mice (6-weeks-old) were purchased from CLEA Japan, Inc. (Tokyo, Japan). All the mice were maintained on a 12-h light/dark cycle in our temperature-controlled central animal facility. All animal experiments were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University (KPU) and were performed according to the Guidelines for Animal Experimentation at KPU.

Evaluation of the gastrointestinal absorption of Zn in KKA^y mice given oral Zn(tanm)$_2$. $^{65}ZnCl_2$ in 0.1 M HCl was obtained from Japan Radioisotope Association Co. (Tokyo, Japan). Prior to use, the $^{65}ZnCl_2$ solution was gently dried using a mantle heater, and the residue was dissolved in DMSO (specific activity = 370 mBq/mL). 15.6 mg $ZnCl_2$ (M.W. = 136.4), 67.7 mg $Zn(alx)_2$ (M.W. = 544.7), or 67.7 mg $Zn(tanm)_2$ (M.W. = 590.3) dissolved in 6.9 mL DMSO was mixed with 220 mBq/600 μL of ^{65}Zn in DMSO solution to exchange the cold Zn with ^{65}Zn. Preparations labelled with ^{65}Zn were stirred at room temperature overnight to exchange the cold Zn with ^{65}Zn. The exchanges were confirmed to be more than 90% by column chromatography. The administered solutions were prepared such that they contained 10 mg Zn/mL (15 mM) and 29.6 mBq ^{65}Zn/mL. The radioactivity administered to each mouse was 296 mBq ^{65}Zn/kg of body weight.

Male KKA^y mice (8-weeks-old) subjected to a 12-h fast were divided into 15 groups (3 compounds with ^{65}Zn compounds × 5 sampling times) and orally administered a single dose of ^{65}Zn-labelled compounds by gavage at a dose of 10 mg Zn/kg of body weight. At 2, 5, 8, 12, and 24 h after the treatment, the mice were sacrificed under ether anesthesia, and blood was collected. The blood was immediately weighed and transferred to polyethylene tubes for gamma counting. Radioactivity due to ^{65}Zn in the blood was measured using the Aloka ARC-360 auto well gamma system (Tokyo, Japan) using the peak area of 1115.5 keV due to the ^{65}Zn nuclide (half-life of ^{65}Zn, 244.26 d). The area under the curve (AUC) of ^{65}Zn levels in the blood was calculated by the bootstrap method [16].

Effects of Zn(tanm)$_2$ in KKA^y mice. Sixteen-week-old KKA^y mice were randomly divided into $Zn(alx)_2$- or $Zn(tanm)_2$-treated, and untreated diabetic mice groups. The untreated normal mice group comprised C57BL/6J mice. Zn(II) complexes or vehicle (polyethylene glycol 400) were administered to the 16-week-old mice at approximately 12:00 AM daily for the following 4 weeks by oral gavage. Dosage of the Zn(II) complexes was 15 mg (229 μmol) Zn/kg/d during the treatment period. Baseline measurements of fed blood glucose level, body weight, and food intake were obtained daily prior to the administrations. Glucose and HbA_{1c} levels in the blood, which was obtained from the tail vein of the mice, were measured by using the glucose oxidase method (Glucocard, Arkray, Kyoto, Japan) and the DCA 2000 system (Bayer Medical Co., Tokyo, Japan), respectively. The blood pressure of the mice was measured by the undirected tail-cuff method using a blood pressure monitor (MK-2000, Muromachi Kikai Co., Kyoto, Japan).

After the 4-week treatment period, the mice were subjected to a 12-h fast, and blood samples were collected by orbital exsanguination under ether anesthesia using heparinized tools (approximately 12:00 AM). The samples were then centrifuged at 5000 rpm for 10 min at 4 °C, and the resulting plasma was used for the analyses of biochemical parameters. Plasma insulin levels were measured using the Glazyme insulin-EIA test (Sanyo Kasei Co., Kyoto, Japan). Plasma leptin and adiponectin levels were measured using ELIZA kits (Quantikine®) purchased from R&D Systems Inc. (Minneapolis, USA). Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined by using the Fuji Dry Chem analyzer (Fuji Medical Co., Tokyo, Japan). Following the collection of blood, the mice were immediately sacrificed under ether anesthesia, and organs such as the right femoral muscle, epididymal fat pads, pancreas, liver, kidney, and right leg bone were removed to determine Zn concentrations.

Tissue samples from the four groups were thoroughly washed using 250 mM sucrose solution to eliminate residual Zn from the body fluid prior to Zn measurement. The samples were weighed and heated repeatedly at approximately 200 °C with 60% HNO_3, 60% $HClO_4$, and 30% H_2O_2 in 50-mL beakers. When the residue became white, the dried samples were dissolved in 1% HNO_3. Then, the Zn concentration was measured using a graphite furnace atomic absorption spectrometer (AAS) (AA-6300 and GFA-EX7i, Shimadzu Co., Kyoto, Japan).

Oral glucose tolerance test (OGTT) in KKA^y mice. After Zn(II) complex treatments, an OGTT was performed. The KKA^y mice were subjected to a 12-h fast, and a dose of 1 g/kg body weight of 1 M glucose solution was orally administered. Blood samples were obtained from the tail vein at 0, 30, 45, 60, 90, and 120 min after glucose loading.

Statistical analysis. All experimental data are expressed as mean values ± standard errors (SE). Statistical analyses were performed using one-way ANOVA 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

^{65}Zn uptake from the gastrointestinal tracts of KKA^y mice orally administered ^{65}Zn-labelled Zn(tanm)$_2$

To examine the gastrointestinal absorption of Zn, type 2 diabetic KKA^y mice were orally administered a single dose of ^{65}Zn-labelled Zn(II) compounds. The ^{65}Zn concentration-time profiles for ^{65}Zn-labelled Zn(II) compounds are shown in Fig. 1. The plasma ^{65}Zn levels in mice administered ^{65}Zn-labelled $Zn(tanm)_2$ ($^{65}Zn(tanm)_2$) were greatly enhanced compared to those of mice administered

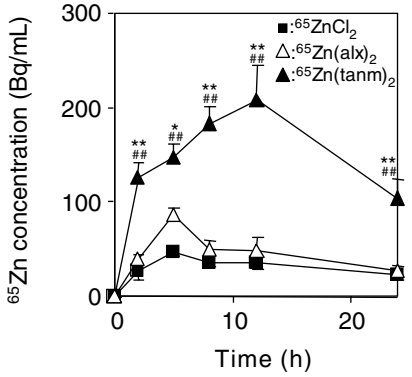

Fig. 1. Blood concentration-time courses of ^{65}Zn in type 2 diabetic KKA^{y} mice after a single oral administration of ^{65}Zn-labelled Zn(II) compounds. Data are expressed as mean values ± SE. Significance: $^{\#\#}p < 0.01$ vs. $^{65}ZnCl_2$; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. $^{65}Zn(alx)_2$.

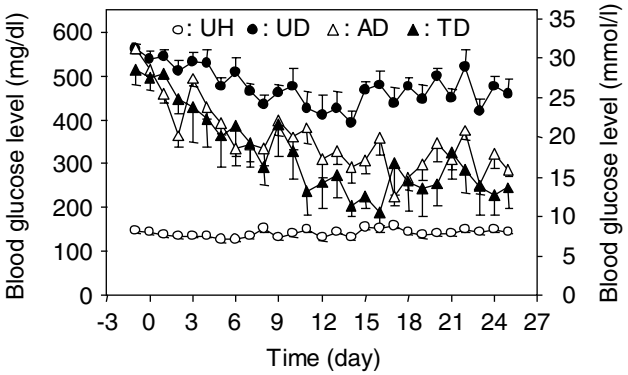

Fig. 2. Changes in blood glucose levels in untreated healthy mice (UH), untreated diabetic mice (UD), Zn(alx)$_2$-treated diabetic mice (AD), and Zn(tanm)$_2$-treated diabetic mice (TD) for 4 weeks. Data are expressed as mean values ± SE for 6 or 7 mice.

^{65}Zn-labelled $ZnCl_2$ ($^{65}ZnCl_2$) or Zn(alx)$_2$ (^{65}Zn(alx)$_2$). Further, the AUC value for ^{65}Zn(tanm)$_2$-treated mice (3671 ± 655 Bq/mL h) was significantly higher than those for $^{65}ZnCl_2$-treated mice (763 ± 130 Bq/mL h) or ^{65}Zn(alx)$_2$-treated mice (1084 ± 210 Bq/mL h), indicating that when Zn is administered as a chemical form of Zn(tanm)$_2$, it is more extensively absorbed from the gastrointestinal tracts than when it is administered as other chemical forms.

Previously, we reported that oral administration of Zn(alx)$_2$ produced a slight hypoglycemic effect in type 2 diabetic KKA^{y} mice [14]. The low activity of Zn(alx)$_2$ was presumed to be due to its low uptake from the gastrointestinal tracts. The stability constant of Zn(tanm)$_2$ ($\log\beta = 22.11$) is higher than that of Zn(alx)$_2$ ($\log\beta = 12.59$), indicating that Zn(tanm)$_2$ is more stable than Zn(alx)$_2$ (see Supplemental information). Because Zn(II) complexes are generally unstable in the presence of proteins occurring in the gastrointestinal tracts, stable Zn(II) complexes such as Zn(tanm)$_2$ might be advantageous since they are extensively absorbed from the gastrointestinal tracts.

Improvement of hyperglycemia, glucose intolerance, and insulin resistance in KKA^{y} mice orally administered Zn(tanm)$_2$

Previously, we reported that Zn(tanm)$_2$ has approximately 14-fold more potent insulin-like activity than Zn(alx)$_2$ in isolated rat adipocytes [15]. In the present study, we observed that the gastrointestinal absorption of Zn when administered as Zn(tanm)$_2$ is much higher than when it is administered in the form of other inorganic Zn(II) salts or Zn(alx)$_2$ in type 2 diabetic mice (Fig. 1). We then examined whether or not orally administered Zn(tanm)$_2$ shows anti-diabetic effects in KKA^{y} mice. When KKA^{y} mice were administered a dose of 15 mg (229 μmol) Zn/kg of body weight of Zn(tanm)$_2$ for 4 weeks, a hypoglycemic effect was observed (Fig. 2). Further, the elevated HbA_{1c} levels were normalized after the oral administrations of Zn(tanm)$_2$ (Table 1). Although a hypoglycemic effect was observed in KKA^{y} mice treated with an identical dose of Zn(alx)$_2$, their HbA_{1c} levels were not improved when compared with those of Zn(tanm)$_2$-treated mice.

In the OGTT performed after the treatment period, the blood glucose levels of the untreated and Zn(alx)$_2$-treated KKA^{y} mice were elevated to the maximum concentration of approximately 360 mg/dL (19 mM) at 15 min after glucose loading. In contrast, the elevation in the blood glucose levels of Zn(tanm)$_2$-treated KKA^{y} mice was suppressed more than in the untreated and Zn(alx)$_2$-treated KKA^{y} mice (Fig. 3). The AUC value for Zn(tanm)$_2$-treated mice (379 ± 47 mg/dL h) was almost equivalent to that for the normal mice (426 ± 13 mg/dL h). The pathogenesis of type 2 diabetes is characterized by insulin resistance in insulin-targeting tissues such as the skeletal muscle, adipose, and liver. The high plasma insulin levels in KKA^{y} mice decreased significantly after treatment with Zn(tanm)$_2$ (Table 1), suggesting that this complex enhanced insulin sensitivity in type 2 diabetes. In contrast, Zn(alx)$_2$ showed no effects on glucose tolerance and insulin sensitivity (Fig. 3 and Table 1). Based on these results, orally administered Zn(tanm)$_2$ was concluded to improve hyperglycemia, glucose intolerance, and insulin resistance in type 2 diabetes.

Improvement of leptin resistance, and increase of depressed plasma adiponectin levels in KKA^{y} mice given oral Zn(tanm)$_2$

Recent advances in molecular and cell biology have shown that adipose tissues not only store excess energy in the form of fat but also secrete biologically active substances called adipocytokines, such as free fatty acids (FFA), tumor necrosis factor-α (TNF-α), resistin, leptin, and adiponectin [17]. Adipocytokines play important roles in maintaining metabolic homeostasis and the development of several diseases such as type 2 diabetes, dyslipidemia, and atherosclerosis [18]. Leptin, an adipocytokine, controls

Table 1
Effects of Zn(II) complexes in KKA^y mice

Parameter	Healthy mice	Diabetic mice		
	Vehicle	Vehicle	$Zn(alx)_2$	$Zn(tanm)_2$
HbA_{1c} (%)	3.5 ± 0.1	$10.2 \pm 0.2^{\#\#}$	$8.3 \pm 0.3^{\#\#,**}$	$3.2 \pm 0.4^{**,\dagger\dagger}$
Plasma insulin level (μU/mL)	7.7 ± 0.8	$22.6 \pm 3.8^{\#}$	$24.5 \pm 7.0^{\#}$	$7.7 \pm 1.6^{*,\dagger}$
Plasma leptin level (ng/mL)	1.9 ± 0.3	$38.2 \pm 2.5^{\#\#}$	$37.7 \pm 2.9^{\#\#}$	$16.0 \pm 2.2^{\#\#,**,\dagger\dagger}$
Body weight (g)	28.8 ± 0.4	$43.3 \pm 1.0^{\#\#}$	$43.9 \pm 0.9^{\#\#}$	$42.3 \pm 1.5^{\#\#}$
Food intake (g)	3.4 ± 0.1	$6.3 \pm 0.3^{\#\#}$	$5.7 \pm 0.2^{\#\#}$	$4.6 \pm 0.2^{\#\#,**,\dagger\dagger}$
Epididymal fat pads weight (g/100 g of body weight)	1.1 ± 0.1	$3.2 \pm 0.1^{\#\#}$	$3.5 \pm 0.2^{\#\#}$	$2.2 \pm 0.2^{\#\#,**,\dagger\dagger}$
Systolic blood pressure (mmHg)	103 ± 2	$118 \pm 2^{\#}$	109 ± 5	$101 \pm 3^{*}$

Data are expressed as mean values ± SE for 5–7 mice.
Significance: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. healthy mice; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. untreated diabetic mice; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. $Zn(alx)_2$-treated diabetic mice.

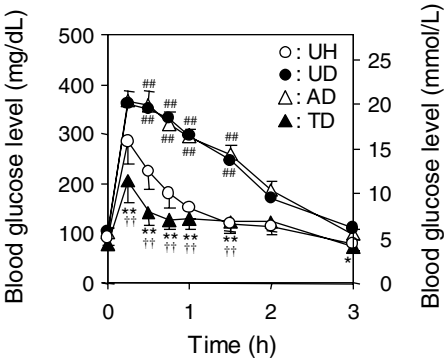

Fig. 3. Effect of $Zn(tanm)_2$ treatment on glucose tolerance in type 2 diabetic KKA^y mice. Changes in blood glucose levels during oral glucose tolerance tests (OGTT). Data are expressed as mean values ± SE for 5–7 mice. UH, untreated healthy mice; UD, untreated diabetic mice; AD, $Zn(alx)_2$-treated diabetic mice; TD, $Zn(tanm)_2$-treated diabetic mice. Significance: $^{\#\#}p < 0.01$ vs. UH; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. UD; $^{\dagger\dagger}p < 0.01$ vs. AD.

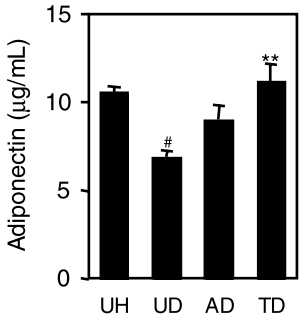

Fig. 4. Effect of $Zn(tanm)_2$ treatment on hypoadiponectinemia in type 2 diabetic KKA^y mice. Data are expressed as mean values ± SE for 5–7 mice. UH, untreated healthy mice; UD, untreated diabetic mice; AD, $Zn(alx)_2$-treated diabetic mice; TD, $Zn(tanm)_2$-treated diabetic mice. Significance: $^{\#}p < 0.05$ vs. UH; $^{**}p < 0.01$ vs. UD.

the overall energy homeostasis via the induction of reduced food intake and promotion of fat loss [19]. Thus, hyperleptinemia observed in KKA^y mice is positively related to the degree of adiposity, suggesting a state of leptin resistance in obesity.

Oral administrations of $Zn(tanm)_2$ significantly reduced hyperleptinemia in KKA^y mice (Table 1), indicating an enhancement in leptin sensitivity. $Zn(tanm)_2$ did not change the body weight of KKA^y mice aged 20 weeks; the excessive food intake and fat weight, however, were significantly suppressed (Table 1). Based on these results, we concluded that $Zn(tanm)_2$ enhanced leptin sensitivity leading to an alleviation of obesity in KKA^y mice.

On the other hand, adiponectin, an adipocytokine, is abundantly present in plasma and leads to enhanced insulin action, suggesting that this adipocytokine maintains insulin sensitivity and glucose homeostasis [20]. However, adiponectin is reduced in patients with increased insulin resistance due to conditions such as obesity, type 2 diabetes, and hypertension [21]. Therefore, this adipocytokine is considered to be a biomarker for developing therapeutics for type 2 diabetes [2]. Previously, we proposed the first example of i.p. injections of a Zn(II) complex with hinokitiol (β-thujaplicin) that enhanced low adiponectin levels in KKA^y mice [22]. In the present study, orally administered $Zn(tanm)_2$ normalized the depressed plasma adiponectin levels in KKA^y mice (Fig. 4). These data suggest a possibility that the increase in plasma adiponectin levels is involved in a mechanism by which Zn(II) complexes improve insulin resistance in KKA^y mice.

Further, daily oral administrations of $Zn(tanm)_2$ for 4 weeks were observed to improve elevated systolic blood pressure (Table 1). The obesity-linked type 2 diabetic KKA^y mice were recently reported to express hypertension due to sympathetic activation mediated by chronic hyperleptinemia [23]. A recent paper also reported that hypoadiponectinemia contributed partially to the development of obesity-linked hypertension such as that in KKA^y mice [24]. Based on these observations, $Zn(tanm)_2$ is considered to improve obesity-linked hypertension by attenuating leptin resistance or hypoadiponectinemia in KKA^y mice.

Organ distribution of Zn in KKA^y mice given oral $Zn(tanm)_2$

After the 4-week treatment period with $Zn(tanm)_2$, Zn levels in the plasma and several tissues were determined

Table 2
Organ distribution of Zn concentration (μg/g wet weight) in KKA^y mice treated with Zn(II) complex

Organ	Healthy mice	Diabetic mice		
	Vehicle	Vehicle	$Zn(alx)_2$	$Zn(tanm)_2$
Plasma	1.26 ± 0.03	$0.93 \pm 0.02^{\#}$	$1.29 \pm 0.03^{*}$	$1.73 \pm 0.23^{\#,**,\dagger}$
Muscle	9.0 ± 0.8	$5.8 \pm 0.5^{\#}$	7.0 ± 0.6	$9.9 \pm 1.2^{*}$
Adipose	2.8 ± 0.2	1.6 ± 0.1	2.7 ± 0.5	$4.0 \pm 0.6^{*}$
Pancreas	32.7 ± 2.1	26.9 ± 1.5	32.4 ± 2.0	$59.2 \pm 7.0^{\#\#,**,\dagger\dagger}$
Liver	20.8 ± 0.8	22.6 ± 0.9	24.3 ± 1.0	26.5 ± 3.1
Kidney	18.2 ± 0.5	17.7 ± 0.6	16.8 ± 0.9	19.4 ± 0.8
Bone	121 ± 4	128 ± 3	$190 \pm 9^{\#\#,**}$	$167 \pm 5^{\#\#,**,\dagger}$

Data are expressed as mean values ± SE for 5–7 mice.
Significance: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. healthy mice; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. untreated diabetic mice; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. $Zn(alx)_2$-treated diabetic mice.

by AAS. The Zn levels observed in the plasma and insulin-targeting tissues such as muscle in KKA^y mice were lower than those of healthy mice (Table 2); this corresponded with other previously reported data [25,26]. When $Zn(tanm)_2$ was orally administered to KKA^y mice for 4 weeks, significant increases in Zn levels were observed in the plasma, muscle, adipose, pancreas, and bone (Table 2). It has been proposed that abnormalities in Zn homeostasis such as Zn deficiency affect glucose metabolism and insulin sensitivity [27]. Such low Zn status in type 2 diabetic patients and animal models may cause impaired Zn absorption in the gastrointestinal tracts and thus affect normal Zn clearance [27–29]. If such abnormal Zn metabolism affects the pathogenesis of type 2 diabetes, alleviating Zn deficiency may treat the diabetic state. Achieving high Zn uptake from the gastrointestinal tracts of animals administered $Zn(tanm)_2$ might be useful in the treatment of diabetes since this would alleviate the Zn deficiency in type 2 diabetes.

In conclusion, orally administered $Zn(tanm)_2$ not only improved hyperglycemia, insulin resistance, and leptin resistance but also increased depressed adiponectin levels in obesity-linked type 2 diabetic KKA^y mice. Although the agents that increase the adiponectin levels are expected to be potential insulin-sensitizing therapeutics, thus far, except for thiazolidinedione derivatives (TDZs), no insulin sensitizers that increase the adiponectin levels have been reported [2]. However, TDZs are associated with edema, body weight gain, and liver dysfunction; these are clinical drawbacks in the treatment of type 2 diabetic patients [2]. In contrast, $Zn(tanm)_2$ did not exhibit such symptoms and no changes in the plasma ALT and AST levels were observed. This indicated that $Zn(tanm)_2$ did not exert appreciable toxic effects in the liver. Hence, we propose $Zn(tanm)_2$ will be an orally active therapeutic for obesity-linked type 2 diabetes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.10.014.

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