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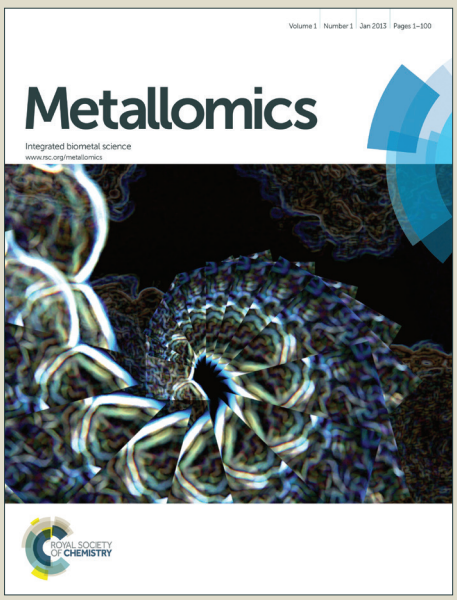

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Morphological analysis of the pancreas and liver in diabetic KK-A^y mice treated with zinc or oxovanadium complexes

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Running head: Metal complexes in KK-A^y mice

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Table of contents entry: Zinc complex has potential antidiabetic applications owing to their protective effects on the morphology of pancreatic islet cells and liver cells in a mouse model of diabetes.

Abstract

The relationship between biometals, such as zinc (Zn^{2+}), vanadium, copper, cobalt, and magnesium ions, and diabetes therapy has been recognized for several years. In particular, the antidiabetic activities of Zn^{2+} and oxovanadium (VO^{2+}) complexes have been measured by biochemical approaches. In the present study, diabetic KK-A^y mice were treated with bis(1-oxy-2-pyridine-thiolato)Zn^{2+} [$Zn(opt)]_2$] and bis(1-oxy-2-pyridine-thiolato)VO^{2+} [$VO(opt)_2$] for 4 weeks, and the antidiabetic activities of these metal complexes were evaluated by biochemical and morphological methods. Additionally, zinc gluconate [$Zn(glc)_2$] and bis(ethylmaltolato) VO^{2+} [$VO(emal)_2$] were used as reference compounds. Pancreatic islet cells were smaller, and there was a tendency towards a lower islet cell area ratio in $Zn(opt)_2$-treated mice compared with nontreated KK-A^y mice. Furthermore, plasma insulin concentrations were significantly reduced to 27.2% of insulin concentrations in nontreated KK-A^y mice. These results suggest that $Zn(opt)_2$ administration provides morphological and biochemical improvements in hyperinsulinaemia. In contrast, in mice that received $Zn(glc)_2$ and VO^{2+} complexes, islet cell size and islet cell area ratio did not differ from those in nontreated controls. $Zn(opt)_2$- and $VO(opt)_2$-treated mice exhibited significantly lower fat deposition and fat deposition area ratio in the liver (63.6% and 65.8% of nontreated KK-A^y mice, respectively) compared to those observed in nontreated KK-A^y mice. The differences in morphological improvements of the pancreas and liver owing to $Zn(opt)_2$ or $VO(opt)_2$ treatment may be explained by differences in the sites of actions of Zn^{2+} and VO^{2+} complexes on different organs in KK-A^y mice. In conclusion, $Zn(opt)_2$ exhibited superior antidiabetic effects than those of $VO(opt)_2$, and this was owing to greater amelioration of morphological parameters of the liver and pancreas.

Introduction

In 2011, an estimated 366 million people worldwide had diabetes mellitus (DM). By 2030, this number is expected to increase to 552 million.[1] The World Health Organization classifies DM as type 1 and type 2; the latter accounts for more than 90% of all patients with DM.[2] Type 2 DM is a chronic metabolic disorder and its prevalence has been steadily increasing worldwide. High blood glucose levels resulting from insulin resistance and impaired insulin secretion is considered a major clinical hallmark of type 2 DM. Various factors such as genetic predisposition, obesity, and lack of exercise have been reported to increase the risk of developing type 2 DM. The condition is treated primarily through exercise and dietary intervention; when these prove ineffective, the use of clinical medicines is initiated. Adverse side effects, such as hypoglycaemia, have been reported for patients in whom the continuous use of medicines leads to difficulty in controlling blood glucose levels. In addition, persistent DM increases the production of reactive oxygen species, leading to oxidative stress. In turn, oxidative stress leads to an increase in the development of DM-specific complications such as nephropathy, retinopathy, and neuropathy.[3,4]

For several years, the role of biometals, such as vanadium, copper, cobalt, magnesium, and zinc (Zn^{2+}) ions, has been recognized in diabetes research. We have previously published evidence of the antidiabetic effect of Zn^{2+} and oxovanadium (VO^{2+}) complexes synthesized with various coordination modes of N_2O_2, N_4, S_4, O_4, and S_2O_2.[5-11] We reported the effects of these complexes on several antidiabetic biomarkers (blood glucose level, haemoglobin A1c, oral glucose tolerance test, and several biochemical parameters). However, we have not previously performed a quantitative morphometrical analysis to evaluate the pharmacological effects of VO^{2+} and Zn^{2+} ions.

KK-A^y, a mouse model of type 2 DM, has been used by several investigators for its diabetic state and because these mice exhibit early-onset and prolonged, severe hyperinsulinaemia.[12,13] In addition, 10-week-old KK-A^y mice develop enlargement of islet cells, increasing islet cell area ratio of tissue section, and a fatty liver.[14] In previous

pharmacological studies in KK-A^y mice, rosiglitazone treatment ameliorated lipid accumulation in islet cells.[15] In contrast, treatment with pioglitazone was associated with distension of hepatocytes and evidence of severe lipid generation.[16] Most morphological characterization studies have used antidiabetic medicines, and there is no evidence in the literature of studies using Zn^{2+} or VO^{2+} complexes.

We previously demonstrated the antidiabetic effect of a 2-week treatment with bis(1-oxy-2-pyridine-thiolato)Zn^{2+} [$Zn(opt)_2$] in KK-A^y mice and a 4-week treatment with bis(1-oxy-2-pyridine- thiolato)VO^{2+} [$VO(opt)_2$] in streptozotocin-induced diabetic rats.[17,18] In the present study, we aimed to examine the pancreas and liver morphological effects of 4-week treatment with oral $Zn(opt)_2$ and $VO(opt)_2$ in KK-A^y. Furthermore, we performed biochemical assays to determine the antidiabetic effects of these complexes. Zinc gluconate [$Zn(glc)_2$], a food additive, and bis(ethylmaltolato) VO^{2+} [$VO(emal)_2$], currently in phase II clinical trials, were used as reference compounds.[19]

Results and discussion

General remarks and behaviour

Abnormal animal behaviour was not observed during the dosing period. Furthermore, body weight and food intake did not change significantly during the 4 weeks of drug administration compared to that in the nontreated KK-A^y mice (Tables 1 and 2).

Glucose-lowering effects of metal complexes

Hyperglycaemia was confirmed in KK-A^y mice. Baseline blood glucose levels ranged between 400 and 600 mg/dL, compared with values of 90–150 mg/dL obtained in nondiabetic C57BL/6J mice.

The effects of oral administration of $VO(opt)_2$, $VO(emal)_2$, $Zn(opt)_2$, and $Zn(glc)_2$ on hyperglycaemia were examined in KK-A^y mice. Daily oral administration of $VO(opt)_2$, $VO(emal)_2$, and $Zn(opt)_2$ reduced blood glucose levels compared with those in the nontreated KK-A^y mice (Fig. 1). The extent of the blood glucose-lowering effect of

these complexes was found to be similar to that reported previously.[17,18] In contrast, daily administration of $Zn(glc)_2$ did not demonstrate a glucose-lowering effect (Fig. 1b).

Effect of metal complexes on biochemical markers

Table 3 shows the results of biochemical analysis in KK-A^y mice. In $VO(opt)_2$-, $VO(emal)_2$-, $Zn(opt)_2$-, and $Zn(glc)_2$-treated groups, plasma concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and triglyceride (TG) were similar to the corresponding concentrations in nontreated KK-A^y mice. In contrast, plasma concentrations of total cholesterol (TCHO) significantly decreased in $VO(opt)_2$-, $VO(emal)_2$-, and $Zn(opt)_2$-treated groups compared to those in the nontreated mice. In addition, plasma concentrations of insulin significantly decreased in mice that received $Zn(opt)_2$. Although there was a tendency for insulin concentrations to be lower in $VO(opt)_2$- and $VO(emal)_2$-treated mice, these changes did not reach statistical significance.

We also measured AST, ALT, TG, TCHO, and insulin levels in nondiabetic C57BL/6J mice. The corresponding values were 38 ± 11 U/L, 27 ± 17 U/L, 71 ± 28 mg/dL, 97 ± 13 mg/dL, and 40.7 ± 24.8 pg/mL, respectively. In the present study, nontreated KK-A^y mice had significantly higher TG, TCHO, and insulin levels compared to the corresponding levels in the nondiabetic C57BL/6J mice.

Although we did not report reduced plasma concentrations of TG, we would expect values to become normalized after continuing treatment with $Zn(opt)_2$ or $VO(opt)_2$. These findings suggest that $Zn(opt)_2$ and $VO(opt)_2$ provide a similar degree of improvement in liver function of KK-A^y mice. We demonstrated that plasma concentrations of TCHO were significantly reduced in $VO(opt)_2$-, $VO(emal)_2$-, and $Zn(opt)_2$-treated mice. VO^{2+} ions, in the form of vanadyl sulphate, have previously been shown to lower plasma TCHO concentrations mainly by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[20] It has been shown

recently that $Zn(opt)_2$ is associated with improvements in TCHO concentrations.[18] Consequently, we hypothesize that, in addition to the HMG-CoA reductase inhibitors, $VO(opt)_2$ and $VO(emal)_2$, $Zn(opt)_2$ has the potential to promote improvements in TCHO.

Histopathological changes in pancreas, liver and testis

Islet cell hypertrophy was observed in 10-week-old and 14-week-old KK-A^y mice compared with C57BL/6J mice from our previous study.[14] Islet cell area ratios of pancreatic tissue sections are shown in Fig. 2. Islet cell area ratio was 1.83 ± 0.62% in 14-week-old KK-A^y mice, compared with 0.72 ± 0.25% in age-matched C57BL/6J control. In the present study, islet cell hypertrophy was characterized by changes in cell shape, with cells either retaining a normal round/oval outline or becoming lobular and irregular (Fig. 3a–b). The regional distribution of cell types was retained in hyperplastic islets, and enlarged islet cells showed no atypia.

Pancreatic islet cells were smaller in $Zn(opt)_2$-treated KK-A^y mice than in nontreated KK-A^y mice (Fig. 3c). Furthermore, there was a tendency for lower islet cell area ratios in $Zn(opt)_2$-treated KK-A^y mice compared with nontreated KK-A^y mice (Fig. 2). However, no change in islet cell size was observed in other treatment groups (Fig. 3d). Oral administration of rosiglitazone in KK-A^y mice has been shown to ameliorate the DM state. This was associated with an amelioration of lipid accumulation in islet cells and normalization of serum insulin concentrations.[15] And then, oral administration of rosiglitazone in Zucker fatty rat has been shown to ameliorate or prevent the islet cell hypertrophy.[21] In addition, the rosiglitazone treatment for 4 weeks in Zucker diabetic fatty rats was found to suppress the inflammatory cell infiltration and fibrosis in islets of Langerhans.[22] In our study, we were able to demonstrate normalization of islet cell ratio and plasma concentrations of insulin in KK-A^y mice treated with $Zn(opt)_2$. Based on these findings, $Zn(opt)_2$ exhibited a similar antidiabetic effect on the pancreas as

rosiglitazone in KK-A^y mice, in terms of recoveries of islet cell size and serum insulin concentration. This effect was due to the potency of $Zn(opt)_2$ to ameliorate both the reduction of plasma insulin concentrations observed in KK-A^y mice and the stress on pancreatic islet cells.

From several previous reports, it was described that the antidiabetic effects induced by vanadate ions or vanadate complexes can be followed on the basis of both the insulin-depend and insulin-independent pathways.[23, 24] On the other hand, as an example of VO^{2+} complexes action in animals, VO^{2+} complexes are known to enhance tyrosine phosphorylation of the insulin receptor β-subunit and the insulin receptor substrate. This is achieved by inhibition of protein tyrosine phosphatase 1B, which in turn activates the signalling pathways of phosphatidylinositol 3-kinase-Akt.[25] In addition, oral administration of VO^{2+} complexes enhances phosphorylation of Akt and glycogen synthase kinase-3β, which are located downstream of the insulin receptor cascade in skeletal muscles.[26] In contrast, Zn^{2+} complexes act at a different site, and this is based on mechanistic investigations of Zn^{2+} ions and Zn^{2+} complexes on Akt phosphorylation, phosphodiesterase inhibition, and alpha-glycosidase inhibition.[27-29] Therefore, Zn^{2+} and VO^{2+} have the potential for different sites of action, which may give rise to different morphological effects as demonstrated by findings from the pancreas in the present study.

Additionally, patients with DM have hyperglycaemia due to insulin resistance or hyperinsulinaemia.[30] The pancreas in a patient with DM must produce greater levels of insulin in order to reduce blood glucose levels. The result is hypertrophy of β-cell islets and increased production of insulin.[31] Since insulin resistance continues for a prolonged period, β-cells become overloaded, and subsequently necrotic. Based on the findings of the present study, $Zn(opt)_2$, but not $VO(opt)_2$, appears to provide a protective effect on the pancreas in the DM state.

Fig. 4 shows the analysis of liver tissue vacuolation area ratios. Fat deposition area

ratios were significantly greater in nontreated KK-A^y mice compared with age-matched C57BL/6J mice. Liver tissue fat deposition area ratios were 1.46 ± 0.49% in 14-week-old KK-A^y mice, and 0.72 ± 0.34% in 14-week old C57BL/6J mice, respectively. Similar fat deposition area ratios were exhibited by Zn(opt)$_2$-treated and VO(opt)$_2$-treated KK-A^y mice, and these values were significantly lower than those observed in nontreated KK-A^y mice.

Greater centrilobular vacuolation of hepatocytes was observed in the liver of 10- and 14-week-old nontreated KK-A^y mice where vacuoles were strongly stained by oil red O, which indicated fat deposition.[14] In the same way, centrilobular vacuolation of hepatocytes and fat diposition were observed in the liver of nontreated 14-week-old KK-Ay mice (Fig.5a-d). In contrast, KK-A^y mice treated with Zn(opt)$_2$ or VO(opt)$_2$ (data not shown) did not exhibit hepatocyte vacuolation (Fig. 5e–f). Treatment with Zn(glc)$_2$-treated (data not shown) and VO(emal)$_2$-treated KK-A^y mice, did not alter fat deposition area ratios from control values (Fig. 5g–h).

In contrast with a previous report of hepatocyte enlargement and severe lipid degeneration in pioglitazone-treated KK-A^y mice,[16] Zn(opt)$_2$ and VO(opt)$_2$ ameliorated fat deposition in hepatocytes. It appears that Zn(opt)$_2$ and VO(opt)$_2$ are not associated with the hepatotoxic effects of pioglitazone.

In addition, we previously reported testicular toxicity associated with VO(opt)$_2$ and VO(emal)$_2$ in KK-A^y mice.[32] These metal complexes were associated with mild-to-severe mineralization and degeneration/necrosis on the basis of morphological analysis (Fig. 6). The toxicity was irreversible. In the present study, both VO(opt)$_2$- and VO(emal)$_2$-treated KK-A^y mice exhibited testicular toxicity. Testicular toxicity is a potential disadvantage associated with the use of VO^{2+} complexes in diabetes treatment. In contrast, testicular toxicity was not observed with Zn^{2+}-complexes (Fig. 6).

Experimental

Reagents

Zinc sulphate ($ZnSO_4$), $Zn(glc)_2$, vanadyl sulphate , ethylmaltol (emal), sodium hydroxide, 1-oxy-2-pyridinethiol, haematoxylin, formalin, disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate, ethanol, acetic acid, isopropanol, glycerine jelly, periodic acid, and sodium bisulphite were purchased from Wako Pure Chemical Co. (Osaka, Japan). Eosin G was obtained from MERCK Co. (Tokyo, Japan). Oil red and phloxine B were obtained from Waldeck GmbH & Co KG (Münster, Germany). All reagents were of analytical or reagent grade and were used without purification.

Synthesis of metal complexes

$VO(opt)_2$ and $Zn(opt)_2$ were prepared by mixing 1-hydroxy-2-pyridinethiol and $VOSO_4$ or $ZnSO_4$ at a molar ratio 2:1 of ligand : metal ion in an aqueous solution of pH 5–6. $VO(emal)_2$ was prepared by mixing emal and $VOSO_4$ at a molar ratio 2:1 of ligand : metal ion in an aqueous solution of pH 5–6.[19] After mixing each solution for 30 min, precipitates were collected, washed several times with pure water, and dried.[17]

Animal preparation

All animal studies were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University (KPU) and were performed according to the Guidelines for Animal Experimentation at KPU. Five-week-old male KK-A^y mice with type 2 DM (n = 26) and male C57BL/6J mice with non-diabetic mouse model (n = 5) were purchased from CLEA Japan Inc. (Tokyo, Japan). Mice were maintained on a 12-h light/dark cycle and housed for 5 weeks in a facility with a room temperature of 23 ± 1 °C and humidity of 60 ± 10% before undergoing experimentation. Mice were allowed free access to solid food (MT, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water.[14]

Animal experiments

Ten-week-old KK-A^y mice received oral administration of $VO(opt)_2$, $VO(emal)_2$, $Zn(opt)_2$ and $Zn(glc)_2$ dissolved in PEG400 or the vehicle only (nontreated) daily at 8 a.m. for 4 weeks. In addition, ten-week-old C57BL/6J mice received oral administration of PEG400 daily at 8 a.m. for 4 weeks. Doses of $VO(opt)_2$ and $VO(emal)_2$ were adjusted to maintain a concentration of 0.38 to 3 mg (7 to 59 μmol) V kg^{-1} body weight/day. Doses of $Zn(opt)_2$ and $Zn(glc)_2$ were adjusted to maintain a concentration of 0.75 to 5 mg (11 to 76 μmol) Zn kg^{-1} body weight/day. The selected doses were the same as those previously shown to lower blood glucose levels in KK-A^y mice, and were much lower than the lethal doses for these metal complexes.[17,18]

All animals were subjected to daily monitoring for overall condition and behaviour. Measurements of physiological data (body weight, and food consumption) were measured once a week at 7 a.m. before treatment.

Biochemical analysis

Blood samples were obtained at the time of sacrifice from the tail vein of mice and subjected to measurement of blood glucose levels using Glucocard (Arkray, Kyoto, Japan). Mice were fasted for 12 h, and blood samples were collected from the cavernous sinus under anaesthesia by heparinised tools. Blood samples were centrifuged at 650 × *g* for 10 min at 4 °C, and the resultant plasma samples were used to analyse various biochemical parameters. Plasma concentrations of AST, ALT, TG, and TCHO were determined by Fuji Dry Chem 4000 (Fuji Medical Co., Tokyo, Japan). Serum levels of insulin were determined by a Rat Insulin ELISA KIT (TMB) (AKRIN-010T, Shibayagi, Gunma, Japan). Biochemical markers in KK-A^y mice were compared statistically with nondiabetic C57BL/6J mice.

Histopathology

Excised pancreas and liver from 14-week-old (n = 4–6) mice were immediately fixed in 10% buffered formalin, sectioned at a thickness of 3 μm, and stained with haematoxylin and eosin (HE) for histopathological examinations. In addition, sequential sections of liver were stained with oil red O. Histopathological assessments were compared with data from age-matched control C57BL/6J mice.

Morphometric analysis

HE-stained tissue sections were scanned with a high-resolution digital slide scanner (NanoZoomer 2.2 Digital Pathology, Hamamatsu Photonics, Hamamatsu, Japan) to prepare digital images. The ndpi image files were opened in colour mode with the NDP.view software (Hamamatsu Photonics). Researchers can calculate easily their selected islet cell area in the digital image, using this software. The islet cell ratio was calculated as [area of islet cells/total area of pancreas specimen]×100%. The area ratio of tissue section of fat deposition was calculated as [area of fat deposition/total area of liver specimen]×100%.

Conclusion

In conclusion, KK-A^y mice treated with $Zn(opt)_2$, $VO(opt)_2$, and $VO(emal)_2$ exhibited improvements in insulin resistance. Treatment with $Zn(opt)_2$ and $VO(opt)_2$ was associated with morphological improvements in the liver. $Zn(opt)_2$ provides further morphological improvements in the pancreas. VO^{2+}- and Zn^{2+}-complexes may act through different pharmacological sites of action on the pancreas and liver. Based on biochemical and morphological analyses, we conclude that $Zn(opt)_2$ has greater potential than $VO(opt)_2$ as an antidiabetic compound.

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Table 1. Body weight of KK-A^y mice at various time points

Group	n	Body weight/g				
		Baseline	1 week	2 weeks	3 weeks	4 weeks
C57BL/6J	5	24.2 ± 2.0	25.1 ± 1.3	25.2 ± 1.4	26.1 ± 1.1	26.9 ± 1.2
Nontreated (control)	4	40.3 ± 3.0	42.5 ± 4.3	42.9 ± 5.0	43.7 ± 4.7	45.1 ± 5.1
$VO(opt)_2$	5	41.2 ± 1.0	39.0 ± 4.0	39.8 ± 3.0	41.5 ± 2.1	43.3 ± 2.3
$VO(emal)_2$	5	40.2 ± 2.5	39.6 ± 3.7	41.2 ± 2.8	42.7 ± 1.8	43.9 ± 2.0
$Zn(opt)_2$	6	42.2 ± 2.4	43.4 ± 2.3	43.0 ± 2.3	44.3 ± 2.1	45.9 ± 2.1
$Zn(glc)_2$	6	41.4 ± 1.9	43.4 ± 2.6	43.6 ± 2.8	44.4 ± 3.3	45.2 ± 3.5

Data are expressed as means ± SD.

$VO(opt)_2$, bis(1-oxy-2-pyridine-thiolato)oxovanadium; $VO(emal)_2$, bis(ethylmaltolato)oxovanadium; $Zn(opt)_2$, bis(1-oxy-2-pyridine-thiolato)zinc; $Zn(glc)_2$, zinc gluconate.

Table 2 Food consumption in KK-A^y mice at various time points

Group	n	Food consumption/g day^{-1}				
		Baseline	1 week	2 weeks	3 weeks	4 weeks
C57BL/6J (g: average of 5 animals)	5	1.8	1.9	2.1	2.4	2.2
Nontreated (control)	4	5.3 ± 2.1	4.4 ± 1.2	4.8 ± 1.0	5.5 ± 1.6	5.3 ± 0.7
$VO(opt)_2$	5	6.0 ± 2.4	4.1 ± 0.6	5.5 ± 0.5	4.8 ± 0.2	4.9 ± 0.2
$VO(emal)_2$	5	5.3 ± 1.5	4.4 ± 1.1	5.1 ± 1.0	5.9 ± 1.3	5.3 ± 2.5
$Zn(opt)_2$	6	5.4 ± 1.6	5.0 ± 0.8	6.7 ± 2.9	5.5 ± 0.4	4.4 ± 0.8
$Zn(glc)_2$	6	5.8 ± 1.4	5.6 ± 0.7	6.2 ± 1.4	5.7 ± 2.2	5.4 ± 0.8

Data are expressed as means ± SD.

$VO(opt)_2$, bis(1-oxy-2-pyridine-thiolato)oxovanadium; $VO(emal)_2$, bis(ethylmaltolato)oxovanadium; $Zn(opt)_2$, bis(1-oxy-2-pyridine-thiolato)zinc; $Zn(glc)_2$, zinc gluconate.

Table 3 Biochemical analysis of nontreated controls and KK-A^y mice treated with metal complexes for 4 weeks

Group	AST (U/L)	ALT (U/L)	TG (mg/dL)	TCHO (mg/dL)	Insulin (pg/mL)
C57BL/6J	38 ± 11	27 ± 17	71 ± 28*	97 ± 13*	40.7 ± 24.8*
Nontreated (control)	50 ± 8	24 ± 2	200 ± 66	175 ± 30	153.5 ± 24.7
$VO(opt)_2$	51 ± 4	22 ± 1	185 ± 66	118 ± 18*	79.4 ± 80.6
$VO(emal)_2$	54 ± 8	22 ± 2	158 ± 38	111 ± 15*	119.2 ± 67.7
$Zn(opt)_2$	56 ± 7	22 ± 2	157 ± 25	130 ± 17*	111.8 ± 13.5*
$Zn(glc)_2$	49 ± 10	29 ± 10	223 ± 63	150 ± 38	263.5 ± 79.5

Data are expressed as means ± SD. *$p < 0.05$ *vs.* nontreated KK-A^y mice (2-tailed Dunnett's test).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; TG, triglyceride; TCHO, total cholesterol; $VO(opt)_2$, bis(1-oxy-2-pyridine-thiolato)oxovanadium; $VO(emal)_2$, bis(ethylmaltolato)oxovanadium; $Zn(opt)_2$, bis(1-oxy-2-pyridine-thiolato)zinc; $Zn(glc)_2$, zinc gluconate.

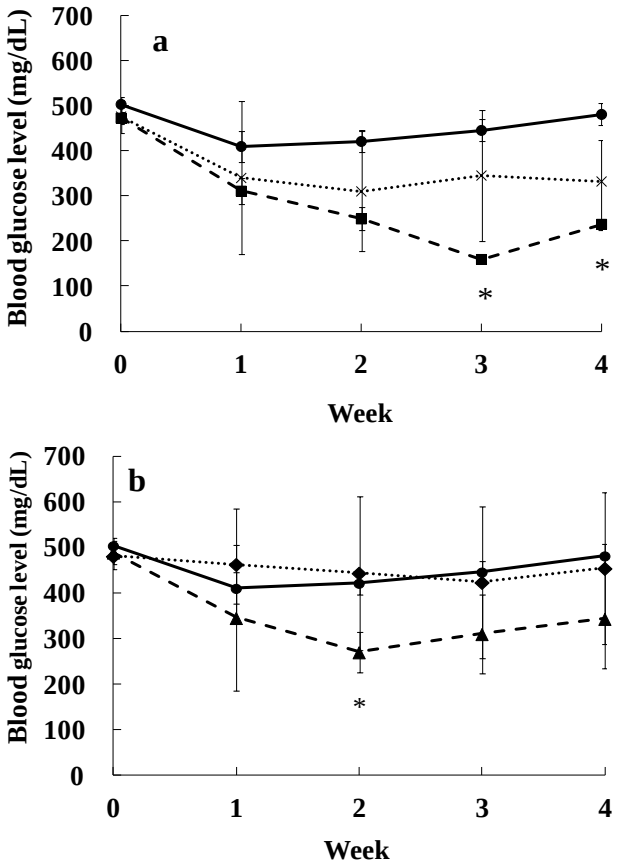

Fig. 1a: blood glucose levels in nontreated KK-A^y mice (● and solid line), and in mice receiving oral administration of $VO(opt)_2$ (■ and dashed line) and $VO(emal)_2$ (× and dotted line) at a dose of 7–59 μmol V kg^{-1} body mass for 4 weeks. b: blood glucose levels in nontreated KK-A^y mice (● and solid line), and in mice receiving oral administration of $Zn(opt)_2$ (▲ and dashed line) and $Zn(glc)_2$ (♦ and dotted line) at a dose of 11–76 μmol Zn kg^{-1} body mass for 4 weeks. Data are expressed as means ± SD. *$p < 0.05$ *vs.* nontreated KK-A^y mice (2-tailed Dunnett's test).

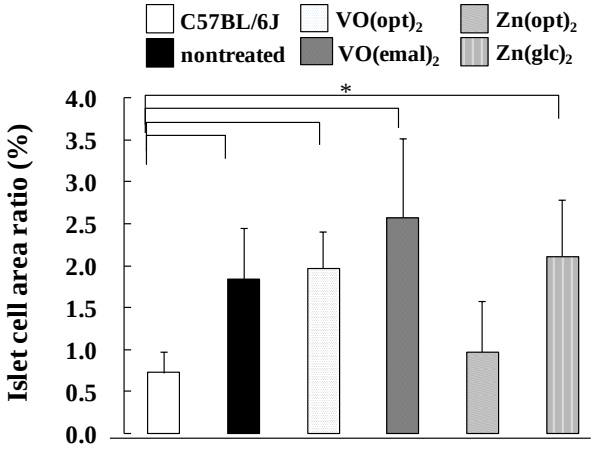

Fig. 2 Islet cell area ratio in pancreas of C57BL/6J mice, nontreated KK-A^y mice, and $VO(opt)_2$-, $VO(emal)_2$-, $Zn(opt)_2$-, and $Zn(glc)_2$-treated 14-week-old KK-A^y mice. Data are expressed as means ± SD. *$p < 0.05$ *vs.* C57BL/6J mice (2-tailed Dunnett's test).

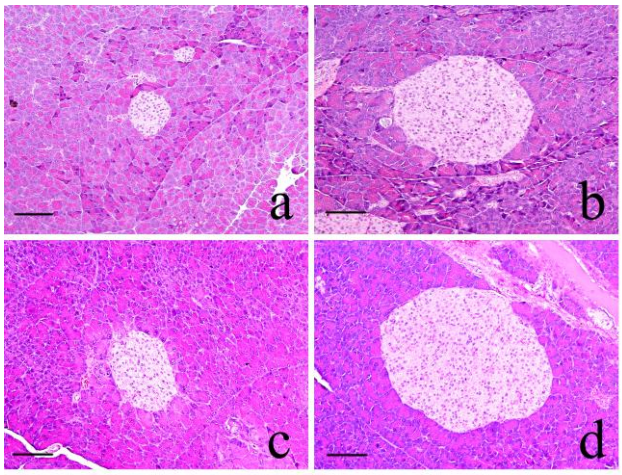

Fig. 3 Islet cells in 14-week-old C57BL/6J (a), nontreated KK-A^y mice (b), KK-A^y mice treated with $Zn(opt)_2$ (c) and $VO(opt)_2$ (d). Islet size and number of islet cells in C57BL/6J mice were within normal range. However, in KK-A^y mice, islet cells were enlarged and the number of islet cells was higher compared with C57BL/6J mice. Islet cells of $Zn(opt)_2$-treated KK-A^y mice were similar in size to islet cells of C57BL/6J mice. Islet cells of $VO(opt)_2$-treated KK-A^y mice were similar in size to islet cells of nontreated KK-A^y mice. Haematoxylin-eosin staining, ×100 (scale bar = 200 μm).

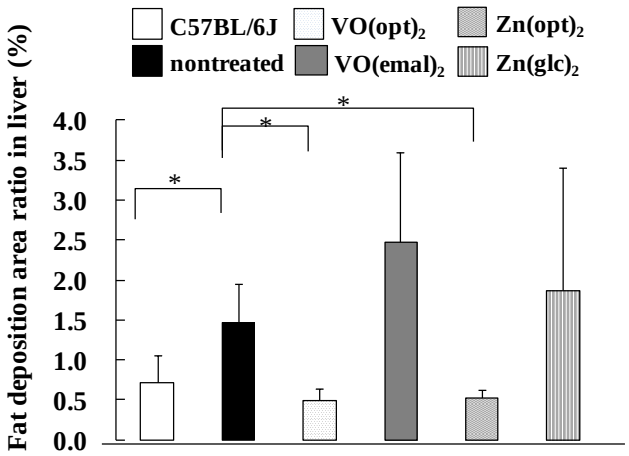

Fig. 4 Fat deposition area ratio in liver of C57BL/6J mice, nontreated KK-A^y mice, $VO(opt)_2$-, $VO(emal)_2$-, $Zn(opt)_2$-, and $Zn(glc)_2$-treated KK-A^y mice. Fat deposition indicated by hepatocellular vacuolation was observed in nontreated KK-A^y mice. Fat deposition area ratio in the liver of nontreated KK-A^y mice was significantly greater when compared with C57BL/6J mice, however, these measures were reversed in $Zn(opt)_2$- and $VO(opt)_2$-treated groups. Data are expressed as means ± SD. *p<0.05 *vs.* nontreated KK-A^y mice (2-tailed Dunnett's test).

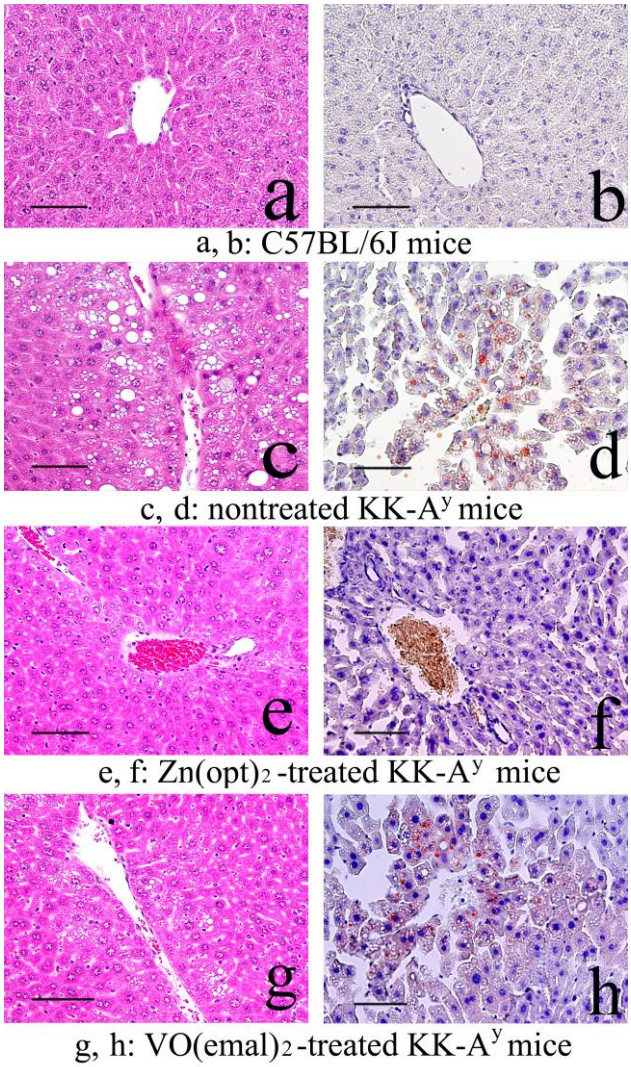

Fig. 5 Hepatic morphology in 14-week-old C57BL/6J mice (a), nontreated KK-A^y mice (c), Zn(opt)$_2$-treated KK-A^y mice (e), and VO(emal)$_2$-treated KK-A^y mice (g). Oil red O-positive hepatocytes were observed in nontreated KK-A^y mice (d) and VO(emal)$_2$-treated KK-A^y mice (h). However, oil red O-negative hepatocytes were observed in Zn(opt)$_2$-treated KK-A^y mice (f), which was also the case in C57BL/6L mice (b). Haematoxylin-eosin staining and oil red O staining, ×200 (scale bar = 100 μm).

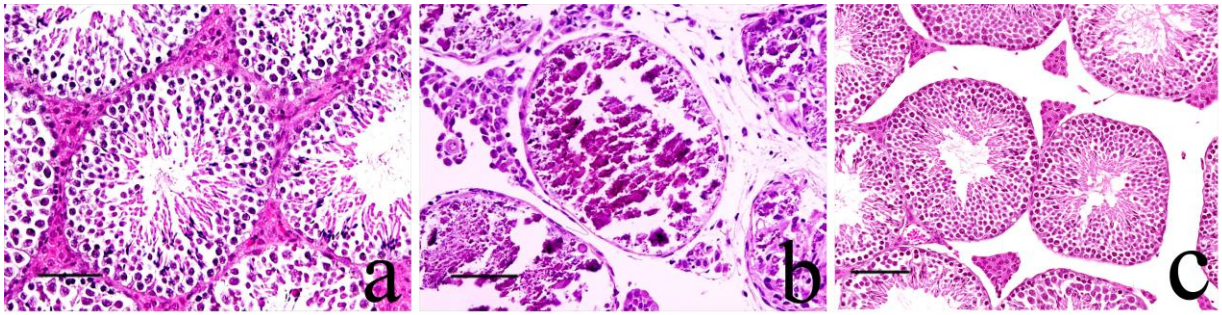

Fig. 6 Testis in 14-week-old nontreated KK-A^y mice (a), $VO(opt)_2$-treated KK-A^y mice (b), and $Zn(opt)_2$-treated KK-A^y mice (c). Haematoxylin-eosin staining, ×200 (scale bar = 100 μm).