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Anti-diabetic effects of vanadium(III, IV, V)–chlorodipicolinate complexes in streptozotocin-induced diabetic rats

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Abstract Vanadium(III, IV, V)–chlorodipicolinate (dipic-Cl) complexes, including $H[V^{III}(dipic\text{-}Cl)_2] \cdot 5H_2O$ (V_3dipic-Cl), $V^{IV}O(dipic\text{-}Cl)(H_2O)_2$ (V_4dipic-Cl) and $K[V^{V}O_2(dipic\text{-}Cl)]$ (V_5dipic-Cl), were prepared with the indicated oxidation states. Our aim was to evaluate the anti-diabetic effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl in streptozotocin-induced diabetic rats. Vanadium complexes were orally administered to diabetic rats at concentrations of 0.1–0.3 mg/ml in the drinking water. We found that vanadium–chlorodipicolinate (V–dipic-Cl) complexes at the concentration of 0.1 mg/ml did not exhibit blood glucose-lowering effects when administered to diabetic rats for 20 days. However, the levels of fasting blood glucose in diabetic rats were decreased after treatment with 0.3 mg/ml of V_4dipic-Cl and V_5dipic-Cl complexes for the following 20 days. Although administration of both V_4dipic-Cl and V_5dipic-Cl significantly lowered diabetic hyperglycemia, the vanadium intake from administration of V_4dipic-Cl is nearly 1.5-fold greater compared to that of V_5dipic-Cl. Treatment with the H_2dipic-Cl ligand and all three V–dipic-Cl complexes significantly lowered serum cholesterol, while administration of the V_5dipic-Cl complex lowered serum cholesterol significantly more than administration of the ligand alone. Treatment with ligand alone did not have an effect on serum triglyceride, while administration of the V_4dipic-Cl and V_5dipic-Cl significantly lowered the elevated serum triglyceride associated with diabetes. Oral administration of the ligand and all V–dipic-Cl complexes did significantly lower diabetes elevated serum alkaline phosphatase. Treatment with H_2dipic-Cl ligand and V_4dipic-Cl and V_5dipicCl significantly lowered diabetes elevated aspartate amino transferase. These results indicate that the health of the treated animals did not seem to be further compromised compared to that of diabetic animals. In addition, oral administration of H_2dipic-Cl, V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl did not alter diabetic serum creatinine and blood urea nitrogen levels, suggesting no significant side effects of vanadium treatment on renal functions at the dose of 0.3 mg/ml in diabetic rats. The results presented here suggest that the anti-diabetic effects of treatment with

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V–dipic-Cl complexes were likely associated in part with the oxidation state of vanadium.

Keywords Vanadium · Chlorodipicolinate · Insulin-enhancing · Lipid · Redox · Diabetes

Introduction

The insulin-mimetic effects of vanadium(V) compounds have been extensively studied both in vivo and in vitro (Reul et al. 1999; Kawabe et al. 2006; Mehdi et al. 2006; Thompson and Orvig 2006; Tracey et al. 2007). Inorganic V salts with different chemical valences (V(IV) and V(V)) significantly lowered the blood glucose of diabetic rats have been reported since the early 1980s by Heyliger et al. (1985) and Ramanadham et al. (1989). However, it has been documented that inorganic V is poorly absorbed from the gastrointestinal (GI) tract, and exhibits severe side effects when orally administrated to diabetic rats (McNeill et al. 1995; Reul et al. 1999; Crans 2000; Srivastava and Mehdi 2004). To decrease the side effects and improve the bioavailability of V in animals, a great amount of organic V compounds with different coordination modes involving $VO(S_2O_2)$, $VO(O_4)$, $VO(N_2S_2)$, and $VO(S_4)$ have been synthesized (Sakurai et al. 2000; Thompson and Orvig 2001; Makinen and Brady 2002). Especially, several vanadyl complexes V(IV), such as BMOV, $VO(pic)_2$ and $VO(acac)_2$, have been reported to exhibit effective and long-term insulin-mimetic activities in experimental diabetic rats by daily intraperitoneal injections or oral administration (Yuen et al. 1993; Sakurai et al. 1995; Amin et al. 2000). The structure activity relationships of vanadyl–picolinic acid complexes have been examined in diabetic rat and mice models and in an in vitro adipocyte insulinomimetic model (Yasui et al. 2002). Also, other oxidation states of V, such as V(III) (Melchior et al. 2001) and V(V) (Crans et al. 2000) have shown promise as potential anti-diabetic agents. To date the exact mechanism(s) of the insulin-enhancing activity of V compounds remains elusive (Marzban et al. 2002).

Some clinical trials with V salts have been carried out in diabetic patients (Cohen et al. 1995; Goldfine et al. 2000; Cusi et al. 2001; Thompson and Orvig 2006). After treatment with vanadyl sulfate at the doses of 150–300 mg/day for 6 weeks, the fasting glucose, hemoglobin A_{1c}, total cholesterol and high density lipoprotein were decreased significantly in type 2 diabetic patients (Goldfine et al. 2000). These studies did not show consistent improvement in insulin sensitivity or carbohydrate oxidation in any of the diabetic populations examined. In 2000, the Phase I clinical trial of bis(ethylmaltolato)oxovanadium(IV) (BEOV) was completed with ethylmaltolato as the ligand in the coordination complex. No dysfunction of liver and kidney as well as abnormity of blood parameters were observed in any of the healthy volunteers (Thompson and Orvig 2006).

Various organic V(III, IV, V) compounds with dipic, dipic-OH or dipic-NH_2 as organic ligand were reported as anti-diabetic agents with little side effects and higher absorption than the simple salts (Crans 2000; Rehder et al. 2002; Crans et al. 2003, 2004; Buglyo et al. 2005; Haratake et al. 2005; Li et al. 2009). Recently, the insulin-enhancing properties of V(III, IV, V)–dipic complexes, including $H[V^{III}(dipic)_2]$, $V^{IV}O(dipic)(H_2O)_2$ (V_4dipic) and $[V^{V}O_2(dipic)]^-$ (V_5dipic) were investigated and the effects of the complexes were found to be different (Buglyo et al. 2005). How halogen substitution would modify the anti-hyperglycemic effect of newly synthesized vanadium(III, IV, V)–chlorodipicolinate complexes (Smee et al. 2009) was investigated. That preliminary study suggested that differences could be found in the anti-diabetic effects of the V chlorodipicolinate complexes (Smee et al. 2009). In this communication, the anti-diabetic effects of daily oral administrations of 0.1–0.3 mg/ml V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl in rats with streptozotocin (STZ)-induced diabetes are described in detail. At the end of the treatment, serum biochemical parameters were used to evaluate the side effects of these V(III, IV, V)–chlorodipicolinate complexes on hepatic and renal functions.

Materials and methods

Materials

Potassium metavanadate (KVO_3), vanadium trichloride (VCl_3) and vanadyl sulfate ($VOSO_4 \cdot xH_2O$) were purchased from Aldrich and used as received.

STZ was obtained from Sigma (St. Louis, MO, USA). Total cholesterol (TCHO), triglyceride (TG), creatinine (Cr), blood urea nitrogen (BUN), aspartate amino transferase (AST), and alkaline phosphatase (ALP) in serum were determined using standard kits from Randox (Antrim, UK). Glucose was assayed using a kit purchased from Beijing Zhongsheng Bioengineering High-technological Company (Beijing, China). All other chemicals used were of analytical grade.

Synthesis of compounds

The H_2dipic-Cl ligand and the $K[V^{V}O_2(dipic\text{-}Cl)]$ (V_5dipic-Cl) complex were prepared as previously described (Ooms et al. 2007). The synthesis of $H[V^{III}(dipic\text{-}Cl)_2] \cdot 5H_2O$ (V_3dipic-Cl) was modified from the preparation of a similar complex (Chatterjee et al. 1998). First 2.56 g (12.7 mmol) of H_2dipic-Cl was dissolved in 200 ml of deionized water. The solution was deaerated by purging with argon for 30 min. To this was added 1.00 g (6.35 mmol) of VCl_3 whereupon a dull yellow precipitate formed. The solution was deaerated for an additional 15 min and the mixture was allowed to stir for 2 h. The yellowish precipitate was filtered off and dried overnight in a vacuum desiccator to give an overall yield of 3.15 g (91% yield). Analytical calculation for $C_{14}H_{15}Cl_2N_2O_{13}V$ was C, 31.07; H, 2.79; N, 5.18 (Found: C, 30.12; H, 2.85; N, 5.99). The synthesis of $V^{IV}O(dipic\text{-}Cl)(H_2O)_2$ (V_4dipic-Cl) started with the addition of 50 ml of water to a mixture of 0.397 g (1.8 mmol) of $VOSO_4 \cdot 3H_2O$ and 0.368 g (1.8 mmol) of H_2dipic-Cl. The pH of the solution was adjusted to 4 with sodium bicarbonate and the solution heated to ~70°C; 20 ml of additional water was added to just dissolve the product. The mixture was then filtered and the filtrate was placed in a refrigerator overnight during which time green plates formed. The plates were filtered off and washed with cold 95% ethanol and dried overnight in air to yield 0.489 g (88%) of V_4dipic-Cl. The product was recrystallized from hot water. Analytical calculation for $C_7H_6ClNO_7V$: C, 27.79; H, 2.00; N, 4.63 (Found: C, 27.98; H, 2.11; N, 4.60). All compounds were synthesized in distilled, deionized water. The schematic structures of H_2dipic-Cl, $H[V^{III}(dipic\text{-}Cl)_2] \cdot 5H_2O$, $V^{IV}O(dipic\text{-}Cl)(H_2O)_2$ and $K[V^{V}O_2(dipic\text{-}Cl)]$ were shown in Fig. 1.

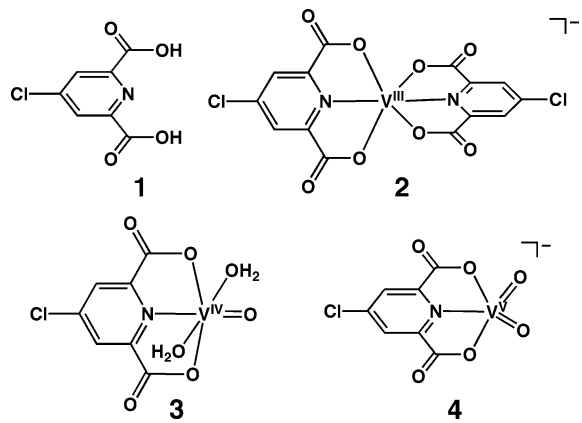

Fig. 1 Schematic structures of H_2dipic-Cl (1), $H[V^{III}(dipic\text{-}Cl)_2] \cdot 5H_2O$ (2), $V^{IV}O(dipic\text{-}Cl)(H_2O)_2$ (3) and $K[V^{V}O_2(dipic\text{-}Cl)]$ (4)

Animals

Male Wistar rats (230–250 g) were obtained from Experimental Animal Center, Peking University Health Science Center, and fed with a standard commercial rat chow. All experiments and protocols described were approved by the Institute of High Energy Physics, Chinese Academy of Sciences. Rats were housed in the animalarium of the Institute of High Energy Physics, Chinese Academy of Sciences and maintained under standardized conditions (12 h light/dark cycle, 24°C) and had free access to food and water. The animals were cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals.

Induction of experimental diabetes

Diabetes was induced in male Wistar rats by a single intravenous (i.v.) injection of freshly prepared STZ (50 mg/kg body weight) in 0.1 mol/l citrate buffer (pH 4.5). The non-diabetic control rats were received only the citrate buffer, intravenously. Animals with fasting (12 h) blood glucose over 12 mmol/l were considered as diabetic. The diabetic rats were randomly assigned into non-treated, H_2dipic-Cl-treated and V-treated diabetic groups. Treatment protocols started 5 days after STZ injection. STZ-induced diabetic rats were given vanadium complexes and the control compound H_2dipic-Cl suspended in distilled water at a concentration of 0.1 mg/ml in the drinking water for the first

20 days after which the concentrations were elevated to 0.3 mg/ml for the following 20 days. Body weight and water intake were monitored daily. In the meantime, the food consumption was recorded. Every 4 days, the blood glucose levels were measured using an Accu-Chek blood glucose meter from a drop of blood obtained by a cut made at the tip of the animal's tail.

Experimental design

This study was a repeated measures parallel design animal experiment with the following groups: control group (Control, $n = 5$), diabetic group (Diabetes, $n = 6$), H_2dipic-Cl group (H_2dipic-Cl, $n = 6$), V_3dipic-Cl group (V_3dipic-Cl, $n = 6$), V_4dipic-Cl group (V_4dipic-Cl, $n = 6$), and V_5dipic-Cl group (V_5dipic-Cl, $n = 6$).

Oral glucose tolerance test (OGTT)

At the end of the experiment, the rats were fasted and gavaged with 2 g/kg body weight of D-glucose (200 mg/ml). The blood samples were collected at 0, 30, 60, 90, and 180 min intervals after the administration of the glucose load, respectively. The blood glucose concentrations were determined as described above.

Biochemical parameters test

Blood was collected from the abdominal vein of each rat with a microsyringe. Serum was separated at 3,000 rpm for 15 min. Biochemical parameters in serum, including TCHO, TG, Cr, BUN, AST, and ALP, were determined using an OLYMPUS AU400 chemistry analyzer.

Statistical analysis

Data were expressed as the mean ± SD. The statistical analyses were performed with one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test for the multiple comparisons among the groups. A P value of less than 0.05 denoted the presence of a statistically significant difference.

Results

Effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl on body weight, food consumption, water intake and vanadium intake in STZ-induced diabetic rats

Streptozotocin is a widely used chemical agent to induce type 1 diabetes, causing the selective destruction of pancreatic β cells (Bennett and Pegg 1981). In this study, STZ-induced diabetic rats presented polyphagia, polydipsia, hyperglycemia and loss of body weight, which is consistent with the previous reports (Yuen et al. 1999; Tan et al. 2005). Figure 2 shows the changes of body weight in control group, diabetic group, H_2dipic-Cl treated group and all the V-treated groups. During the whole experimental period, the body weights of the control rats were gradually and significantly elevated. However, the body weights of STZ-induced diabetic rats were significantly lower than those of the controls. Moreover, there were no significant differences in body weight among the diabetic rats, the H_2dipic-Cl treated rats, and all the other V-treated rats. Diabetes is known to cause weight loss, however, oral administration of V compounds or ligand in the drinking water at concentrations of 0.1 or 0.3 mg/ml did not significantly further decrease the body weight of the diabetic rats. In STZ-induced diabetic rats, insulin deficiency was reported to cause weight loss, probably due to poor utilization of nutrients and muscle protein degeneration, which was reversed by insulin treatment (Wei et al. 2007). The fact that diabetic

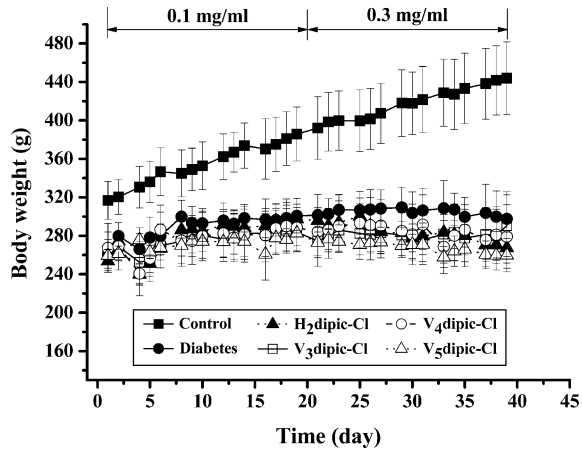

Fig. 2 Changes of body weight in experimental groups. Values are expressed as mean ± SD, $n = 6$

Table 1 Food consumption and water intake after vanadium treatment at the concentrations of 0.1 and 0.3 mg/ml in experimental groups

	Food consumption (g/rat/day)		Water intake (ml/rat/day)	
	0.1 mg/ml	0.3 mg/ml	0.1 mg/ml	0.3 mg/ml
Control	28.2 ± 5.1***	28.5 ± 1.4***	67 ± 8***	73 ± 15***
Diabetes	46.3 ± 4.6	46.5 ± 2.1	238 ± 24	215 ± 23
H_2dipic-Cl	46.2 ± 3.8	40.7 ± 3.8**	239 ± 35	180 ± 20***
V_3dipic-Cl	47.6 ± 4.7	40.9 ± 3.5**	238 ± 27	191 ± 23**
V_4dipic-Cl	45.2 ± 3.4	39.9 ± 2.7**	233 ± 27	157 ± 27***
V_5dipic-Cl	42.7 ± 6.8	33.4 ± 4.7***	176 ± 29***	103 ± 20***

Values are expressed as mean ± SD, $n = 5$–6, ** $P < 0.01$ and *** $P < 0.001$ vs. diabetic groups

weight loss was not reversed by V compounds in these experiments, suggests that the influence of V on protein and lipid metabolisms is different from that of insulin in vivo in this study.

Diabetic rats exhibited significant increases in food consumption ($P < 0.001$) and water intake ($P < 0.001$) compared to those of the controls (Table 1). When diabetic rats were treated with V compounds at a concentration of 0.1 mg/ml in the drinking water, there were no significant differences in food consumption among the diabetic group, the H_2dipic-Cl group and all the V-treated groups. We found that water intake of V_5dipic-Cl treated rats was significantly decreased compared to that of diabetic rats at the concentration of 0.1 mg/ml. In contrast, when the concentration of V compound in the drinking water was elevated to 0.3 mg/ml, the food consumption and water intake significantly declined in both the H_2dipic-Cl group and the V-treated groups compared to the diabetic group. These results show that the higher dose of V intake ameliorated the symptoms of polyphagia and polydipsia in this diabetic animal model. These results are consistent with previous observations made during treatment with unpleasant tasting solutions of vanadium compounds. The unpleasant taste of the solutions would result in lower consumptions and must be considered in interpreting the results.

In addition, elemental V intake from the drinking water was calculated per kg/day and shown in Fig. 3. With 0.1 mg/ml V complex in the drinking water, the average V intake in the group treated with V_4dipic-Cl group was markedly higher than that in the V_3dipic-Cl treated group (12.9 ± 3.2 vs. 7.7 ± 1.9 mg/kg/day, $P < 0.001$) and the V_5dipic-Cl group (12.9 ± 3.2 vs. 8.6 ± 2.0 mg/kg/day, $P < 0.001$). A similar trend

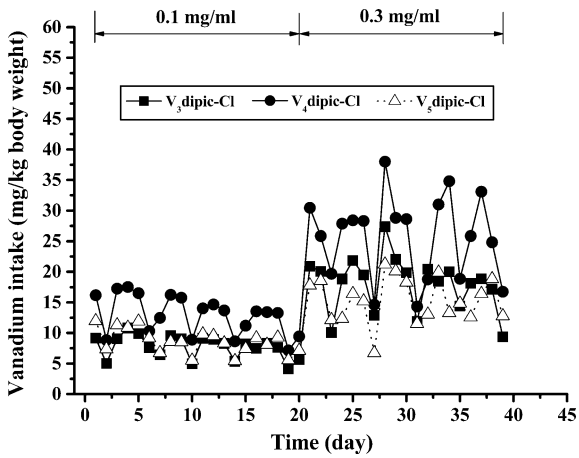

Fig. 3 Vanadium intake from drinking water in V_3dipic-Cl treated group, V_4dipic-Cl treated group and V_5dipic-Cl treated group

was observed when 0.3 mg/ml of V_4dipic-Cl was in the drinking water as well. V intake of the V_4dipic-Cl treated rats is significantly elevated compared to that of the V_3dipic-Cl treated rats (25.7 ± 6.8 vs. 18.0 ± 4.5 mg/kg/day, $P < 0.001$) and V_5dipic-Cl treated diabetic rats (25.7 ± 6.8 vs. 15.4 ± 3.7 mg/kg/day, $P < 0.001$). However, there was no significant difference in V intake between the V_3dipic-Cl treated group and V_5dipic-Cl treated group at either concentration of V in the drinking water.

Effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl on blood glucose levels and oral glucose tolerance test (OGTT) in STZ-induced diabetic rats

Fasting blood glucose was monitored for 40 days after the start of treatment with V complexes in all the animals on day 3, 7, 11, 15, 20, 24, 28, 32, 36, and 40

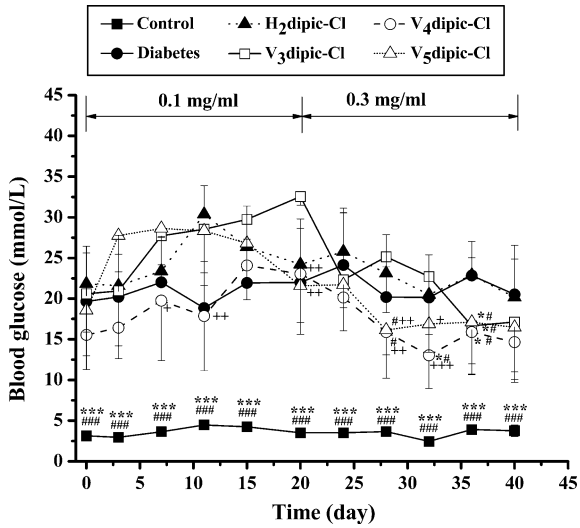

Fig. 4 Blood glucose levels in experimental groups. Values are expressed as mean ± SD, $n = 5$–6, $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. diabetic group; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ vs. H_2dipic-Cl treated group; $^{+}P < 0.05$, $^{++}P < 0.01$ and $^{+++}P < 0.001$, V_4dipic-Cl treated group and V_5dipic-Cl treated group vs. V_3dipic-Cl treated group

(Fig. 4). Intravenous administration of STZ (50 mg/kg, i.v.) produced a significant increase in blood glucose levels in diabetic rats compared to the controls ($P < 0.001$). V(III, IV, V)–chlorodipicolinate complexes administered at the concentration of 0.1 mg/ml in the drinking water did not show glucose-lowering effects. However, when the concentration of V compounds in the drinking water was elevated to 0.3 mg/ml, the levels of blood glucose in the V-treated groups were gradually reduced. At both concentrations of compound administration of the H_2dipic-Cl ligand did not cause blood glucose levels to drop. At day 28, the blood glucose levels in the V_4dipic-Cl treated group and V_5dipic-Cl treated group were significantly lower than those in the H_2dipic-Cl treated group ($P < 0.05$). In the next 4 days, the blood glucose level of the V_4dipic-Cl treated rats was significantly lower compared to both diabetic and H_2dipic-Cl treated rats ($P < 0.05$). At day 36, the concentration of blood glucose in all the V-treated groups were significantly decreased compared to those of the diabetic group and H_2dipic-Cl treated group ($P < 0.05$). Animals treated with 0.1 mg/ml V_3dipic-Cl in the drinking water showed the highest blood glucose levels, while after exposure to 0.3 mg/ml complex blood glucose levels started to drop in this group. At day 20, 28 and 32, the blood glucose levels in V_4dipic-Cl treated group and V_5dipic-Cl treated group were significantly lower compared to those of the V_3dipic-Cl treated group, while at the end of the experiment at day 36 and 40 the lowered blood glucose levels of animals treated with all V complexes compared to the diabetic animals were indistinguishable from each other. These results demonstrate the anti-diabetic effects of V(III, IV, V)–chlorodipicolinate complexes in a dose-dependent manner. Moreover, the V complexes showed an oxidation state dependent effect on blood glucose levels in this experiment. During treatment with 0.3 mg/ml V complexes, blood glucose levels in the V_4dipic-Cl and V_5dipic-Cl treated animals were lowered sooner than those in the animals treated with V_3dipic-Cl.

At the end of the experiment, an oral glucose tolerance test (OGTT) in diabetic rats was carried out. After oral administration of 2 g D-glucose/kg body weight to all rats, blood glucose levels increased to the maximum at 30 min, and then gradually decreased. The OGTT showed a non-statistical trend of improvement of glucose tolerance in the challenge portion of the experiment with V_3dipic-Cl and V_5dipic-Cl treatment, while lower blood glucose levels were observed in the final portion of the experiment for the V_4dipic-Cl and V_5dipic-Cl treated animals (data not shown). A study with more animals would be needed to test the hypothesis that V(III, IV, V)–chlorodipicolinate complexes at the concentration used can improve the glucose tolerance by the OGTT test.

Effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl on diabetic dyslipidemia in STZ-induced diabetic rats

Dyslipidemia, which can range from hypercholesterolemia to hyperlipoproteinemia, is a frequent complication in all types of diabetes (Biesenbach 1989; Gylling et al. 2004). The largest class of diabetes altered gene expression returned to normal levels by vanadyl sulfate treatment involved genes from major lipid biosynthetic pathways in gene expression studies using DNA microarrays (Willsky et al. 2006). In the present study, we determined the concentrations of total cholesterol (TCHO) and triglyceride (TG) in serum. As expected both serum TCHO and TG

concentrations were elevated in the diabetic group compared to those in the control group. TCHO and TG levels were increased by 42% and 40%, respectively.

There was significant lowering of the elevated TCHO of the diabetic animals when compared to TCHO levels in the rats treated with the H_2dipic-Cl ligand ($P < 0.05$), V_3dipic-Cl ($P < 0.01$), V_4dipic-Cl ($P < 0.001$) or V_5dipic-Cl ($P < 0.001$) as seen in Fig. 5a. Furthermore, TCHO concentrations in the V_5dipic-Cl treated rats were significantly lower than the TCHO in the H_2dipic-Cl treated group ($P < 0.01$). These results show different effects of treatment with V–dipic-Cl complexes in different oxidation states. The treatment with the H_2dipic-Cl ligand or the V–dipic-Cl complexes had a synergistic effect on lowering TCHO in this experiment, demonstrating an effect of a V–dipic-Cl complex in lowering TCHO in this experiment.

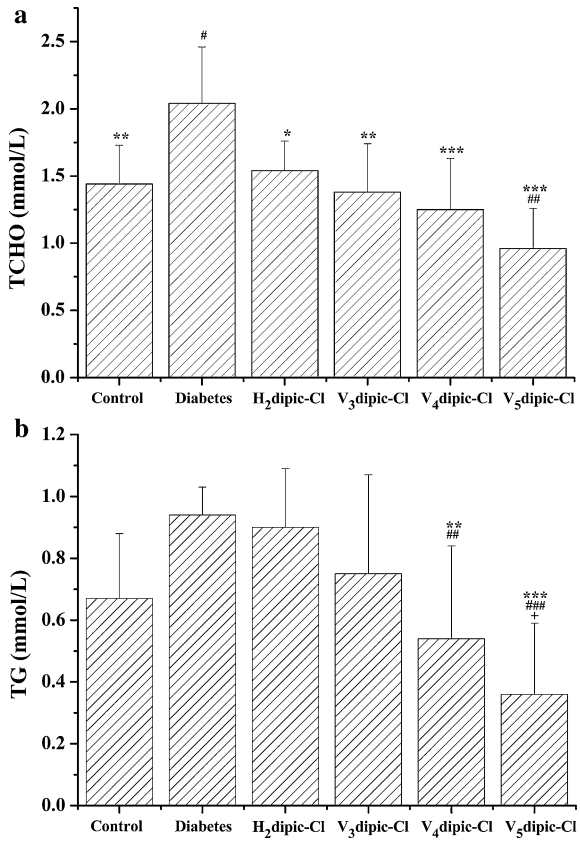

Fig. 5 Anti-hyperlipidemia effects of V_4dipic-Cl and V_5dipic-Cl in STZ-diabetic rats. Serum concentrations of TCHO (**a**) and TG (**b**) were determined in experimental groups. Values are expressed as the mean ± SD, $n = 5$–6, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. diabetic group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. H_2dipic-Cl treated group; $^{+}P < 0.05$, V_5dipic-Cl treated group vs. V_3dipic-Cl treated group

The elevated serum TG level in diabetic rats was significantly lowered after treatment with V_4dipic-Cl ($P < 0.01$) and V_5dipic-Cl ($P < 0.001$) and not with V_3dipic-Cl as seen in Fig. 5b. There was no significant lowering of the elevated TG level in diabetic rats by the H_2dipic-Cl ligand alone. The lowered TG levels of the rats treated with the V_4dipic-Cl and V_5dipic-Cl complexes were also significantly different than those treated with the H_2dipic-Cl ligand ($P < 0.01$, $P < 0.001$, respectively), which would be expected since treatment with H_2dipic-Cl ligand did not lower the elevated TG levels of diabetes. The TG levels in the V_5dipic-Cl treated group were the lowest and these levels were also significantly lower than that in the V_3dipic-Cl treated group ($P < 0.05$). Since the H_2dipic-Cl ligand alone did not lower serum TG, the oxidation state dependent lowering of serum TG by treatment with V–dipic-Cl complexes was easier to visualize than in the results reported here for the lowering of serum TCHO.

Administration of V–dipic-Cl complexes was beneficial in correcting diabetic hyperlipidemia in these animals as seen by our results measuring serum TG and TCHO. The anti-hyperlipidemic effects of the V–dipic-Cl complexes, similarly to the anti-hyperglycemic effects reported above, varied with the oxidation state of the complexes. Oxidation–reduction interactions of the V complexes with cellular metabolism may be responsible for some of the anti-diabetic metabolic effects of the V–dipic-Cl complexes observed here for both lipid metabolism and glucose homeostasis.

Effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl on serum biochemical analyses in STZ-induced diabetic rats

It has been previously reported that hyperglycemia is associated with liver dysfunction in type 1 diabetes. Typical serum biochemical parameters, such as AST and ALP, are often tested to evaluate whether the liver is damaged or diseased. When the liver is not functioning properly, the levels of the above enzymes will be elevated and this has been previously reported in type 1 diabetes (Arkkila et al. 2001; Nannipieri et al. 2005). Therefore, the activities of serum AST

and ALP in diabetic rats were measured in this study (Table 2). After 40 days of treatment the activity of AST in serum was not significantly lowered in diabetic rats, while the activity of ALP was significantly lower ($P < 0.001$). The activities of both AST and ALP were significantly decreased in the H_2dipic-Cl treated group ($P < 0.01$), V_4dipic-Cl treated group ($P < 0.01$) and V_5dipic-Cl ($P < 0.05$) treated group compared to those in the diabetic group. Moreover, the activity of only ALP was significantly decreased after treatment with V_3dipic-Cl ($P < 0.01$). The results suggest that V–dipic-Cl complexes may be capable of ameliorating the impaired liver function in STZ-diabetic rats, which is consisted with previously reported results for treatment with vanadyl sulfate (Koyuturk et al. 2005).

Furthermore, we evaluated the effects of V administration on renal function in STZ-induced diabetic rats. The serum Cr and BUN are good indicators of renal function. If kidney function falls, the levels of Cr and BUN will rise. Thus, serum concentrations of Cr and BUN were determined after 40 days of treatment in this study (Table 2). Only the BUN levels were significantly elevated in the diabetic animals compared to the controls ($P < 0.01$). Oral administration of V–dipic-Cl complexes did not alter serum Cr and BUN levels compared to the diabetic rats. The results indicate that administration of V complexes do not lead to side effects on renal function at the concentrations of 0.1–0.3 mg/ml.

Discussion

Taken together, the anti-diabetic effects of the V–dipic-Cl complex were oxidation state specific in this study. The anti-diabetic effects of V_4dipic-Cl and V_5dipic-Cl were more pronounced compared to those of V_3dipic-Cl in the diabetic rats. Although V_4dipic-Cl showed the same effects in lowering blood glucose and improving the glucose tolerance compared to V_5dipic-Cl, the V intake of V_4dipic-Cl from the drinking water was higher than that of V_5dipic-Cl (about 1.5-fold). These results imply that the insulin-enhancing property of V_5dipic-Cl is more effective than that of V_4dipic-Cl, which is consistent with the previous studies with the parent dipicolinate complexes (Buglyo et al. 2005; Haratake et al. 2005).

Further work with the V dipicolinic acid complexes is needed to determine any non oxidation state dependent properties of the V_3dipic-Cl complexes that might lower the insulin-enhancing activity of this type of molecule. Factors to be considered include increased size, caused by the presence of two ligands, one coordinated to each V, or inherent decreased chemical stability. Oxidation state differences are observed between the anti-diabetic action of the V_4dipic and V_5dipic complexes reported here with the addition of the Cl group and have previously been reported in the absence of the Cl group (Buglyo et al. 2005). Even if the V_3dipic complex turns out to have decreased insulin-enhancing activity for some other specific reason not related to its oxidation state, the results accumulating from studies using V_4dipic and V_5dipic complexes, that have similar size and stability, implicate the interactions of these V complexes with cellular oxidation–reduction processes as an important component in the anti-diabetic action of these compounds.

Many studies have demonstrated that V complexes with various oxidation states cause different anti-diabetic effects (Crans 2000; Rehder et al. 2002; Crans et al. 2003, 2004; Buglyo et al. 2005; Haratake et al. 2005). Among V_3dipic, V_4dipic and V_5dipic

Table 2 Effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl on serum biochemical parameters in STZ-induced diabetic rats

	Cr (mmol/l)	BUN (mmol/l)	AST (U/l)	ALP (U/l)
Control	56 ± 5	4.5 ± 0.6**,###	216 ± 33	63 ± 7***,##
Diabetes	46 ± 12	12.6 ± 3.0	257 ± 133##	747 ± 326###
H_2dipic-Cl	48 ± 2	12.0 ± 2.5	147 ± 14**	356 ± 146***
V_3dipic-Cl	57 ± 10	13.5 ± 5.8	200 ± 46	358 ± 130**
V_4dipic-Cl	45 ± 4	10.6 ± 2.2	146 ± 17**	270 ± 84***
V_5dipic-Cl	52 ± 9	14.6 ± 3.6	170 ± 44*	212 ± 53***

Values are expressed as mean ± SD, $n = 5$–6, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. diabetic group; ## $P < 0.01$ and ### $P < 0.001$ vs. H_2dipic-Cl group

complexes, the V_5dipic complex seems to be more effective than V_3dipic and V_4dipic at lowering high blood glucose in diabetic animals (Buglyo et al. 2005). In addition, insulin-mimetic activities of V hydroxamic acid complexes were observed in STZ-induced diabetic mice. After a single intraperitoneal injection, the V(V) exhibited was more effective at lowering diabetic hyperglycemia than V(IV) (Haratake et al. 2005). Based on the present and previous studies, we suspect that the anti-diabetic effects of V(III, IV, V)–chlorodipicolinate complexes are related to the oxidation state of V. It has been reported that V(V) is more stable at pH 2–5 than in the neutral condition (Crans 2000). Thus, V(V) is better able to get though the acidic conditions of the stomach. As V(V) reaches to intestine or blood, the stability will be decreased (Crans 2000) and the V(V) may be reduced to V(IV). Since V(III) is not stable in vivo, the biotransformation process of V compounds may affect their anti-diabetic potentials in vivo.

Diabetes is a multifactorial disease and it should not be surprising to find more than one mechanism involved in the insulin-enhancing activity of V complexes. The results presented here show an oxidation state dependence for the insulin-enhancing activity of V–dipic-Cl complexes. Therefore, we speculate that in addition to possible inhibition of PTP1B by V–dipic-Cl complexes in vivo, interactions with cellular oxidation–reduction pathways may play an important role in the anti-diabetic activities of the V–chlorodipicolinate complexes.

In conclusion, we investigated the anti-diabetic effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl in STZ-induced diabetic rats. All the V compounds were orally administrated in drinking water at the concentrations of 0.1–0.3 mg/ml. The anti-diabetic effects of V_3dipic-Cl, V_4dipic-Cl and V_5dipic-Cl were observed at the concentration of 0.3 mg/ml V compounds ameliorating both the hyperglycemia and hyperlipidemia of diabetes. Administration of V_4dipic-Cl or V_5dipic-Cl was more effective than administration of V_3dipic-Cl. In contrast, elemental V intake from the drinking water in V_5dipic-Cl group was significantly lower than that in V_4dipic-Cl group. These results suggest that the anti-diabetic effect of V_5dipic-Cl is more potent than that of V_4dipic-Cl. Hepatic functions in STZ-induced diabetic rats appeared to be slightly improved after 40 days treatment with V_4dipic-Cl and V_5dipic-Cl. No negative side effects of treatment with V(III, IV, V)–chlorodipicolinate complexes on renal functions in diabetic rats were observed at the end of the experiment. Further studies concerning the stabilities of V complexes in vivo, the absorption rate in GI tract and the toxicity in animals need to be carried out. It will remain for future studies to elucidate the exact mechanism of the anti-diabetic effects of V(III, IV, V)–chlorodipicolinate complexes to expand our knowledge concerning the applications of V compounds in the treatment of diabetes mellitus.

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