Anti-diabetic effects of sodium 4-amino-2,6-dipicolinatodioxovanadium(V) dihydrate in streptozotocin-induced diabetic rats

Ming Li, Jason J. Smee, Wenjun Ding, Debbie C. Crans

Abstract

The evaluation of the anti-diabetic effects of an organic vanadium(V) complex in streptozotocin (STZ)-induced diabetic rats was investigated. The STZ-induced diabetic rats were orally administrated with sodium 4-amino-2,6-dipicolinatodioxovanadium(V) dihydrate (V5dipic-NH2), a vanadium(V) coordination compound. The compound was administered through drinking water at a concentration of 0.1 mg/mL for 20 days, and then the concentration was increased to 0.3 mg/mL for the following 20 days. At the end of the experiment, V5dipic-NH2 statistically significantly reduced the levels of blood glucose (P < 0.01), serum total cholesterol (P < 0.01), triglycerides (P < 0.01) and the activities of serum aspartate aminotransferase (P < 0.05) and alkaline phosphatase (P < 0.01) compared to untreated diabetic animals. In addition, the daily intake of elemental vanadium was markedly decreased in V5dipic-NH2-treated diabetic rats compared to vanadyl sulfate (VOSO4)−treated diabetic rats, which suggested that the anti-diabetic activity of the element vanadium was elevated after being modified with an organic ligand. These results suggested that V5dipic-NH2, as an organic vanadium compound, is more effective than inorganic vanadium salt at alleviating the symptoms of diabetes.

1. Introduction

Diabetes mellitus is a progressive complication causing serious health problems to human beings. It is characterized by chronic hyperglycemia, disorders of carbohydrate and lipid metabolism, and microvascular pathology in the retina, renal glomerulus and peripheral nerves [1]. Therefore, numerous therapies in diabetes patients have been developed to maintain the normal level of blood glucose [2–5]. Vanadium-containing compounds are generally regarded to have insulin-enhancing properties and anti-diabetic effects both in vivo and in vitro [4–8]. Several studies have shown that vanadium compounds enhance glucose transport, stimulate glycogen and lipid synthesis, and inhibit gluconeogenesis and lipolysis in isolated cells and animal models [9–11]. Inhibition of protein tyrosine phosphatase (PTPase) activity by vanadium has been generally considered as one of the modes of action [12,13]. More recently, organically-chelated vanadium compounds, such as bis(maltolato)oxovanadium(IV) (BMMOV) [14], vanadyl-poly(γ-glutamic acid) complex (VO-γ-PGA) [2] and a range of other vanadium complexes [15,16], have been synthesised and demonstrated to be effective in lowering hyperglycemia [17]. Perhaps most importantly in these studies is the fact that these compounds decrease the side effects of inorganic vanadium salts, possibly by enhancing their rates of absorption in streptozotocin (STZ)-induced diabetic rats [17]. Since dipicolinate vanadium compounds were recently found to be effective insulin-enhancing agents [18,19], we were interested in exploring modified dipicolinato-oxovanadium(V) compounds, which are known to be very potent phosphatase inhibitors [20].

The vanadium(V)-containing compounds p-aminodipicolinato-dioxovanadium(V) (V5dipic-NH2, Fig. 1) is a derivative of the parent dipicolinatodioxovanadium(V) complex [13,21], a well-known compound with insulin-enhancing properties. The present study was performed to investigate the anti-diabetic effects of V5dipic-NH2 in STZ-induced diabetic rats, in order to examine if the amino substituent affects the insulin-enhancing properties of this type of complex. Moreover, we evaluated the biochemical parameters in the serum of diabetic rats.

2. Materials and methods

2.1. Materials

STZ was obtained from Sigma (St. Louis, MO, USA). Vanadyl sulfate (VOSO4·3.5H2O) was purchased from Aldrich Chemical...
Company. Total cholesterol (TCHO), triglycerides (TG), creatinine (CR), blood urea nitrogen (BUN), aspartate amino transferase (AST), and alkaline phosphorase (ALP) in serum were determined using standard kits from Randox (Antrim, UK). Accu-Chek blood glucose monitor was obtained from Roche (Roche Diagnostics GmbH, Mannheim, Germany). All other chemicals used were of analytical grade.

2.2. Synthesis and characterization of V5dipic-NH2

The V5dipic-NH2 complex was prepared as the sodium salt, Na[VO2(dipic-NH2)]·2H2O, according to a previously reported procedure [22]. The compound was checked for purity by 1H and 51V NMR and elemental analysis. The results were found to be in agreement with those reported previously [23].

2.3. Experimental procedures

Male Wistar rats, weighing about 230–250 g, were obtained from the Experimental Animal Center, Peking University Health Science Center. 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 standard, solid food and water. The animals were cared for in accordance with the principle of the Guide for Care and Use of Experimental Animals.

Diabetes was induced by a single intravenous injection of STZ (50 mg/kg body weight) in 0.1 mol/L citrate buffer (pH 4.5). The control rats were injected with an equal volume of citrate buffer alone. Rats with a glucose level higher than 13.3 mM were considered to be diabetic.

V5dipic-NH2 and VOSO4 were orally administered to STZ-induced diabetic rats in drinking water at a concentration of 0.1 mg/mL for 20 days, and then elevated to 0.3 mg/mL for the following 20 days. All of the solutions were freshly prepared prior to administration.

Normal and diabetic rats were randomly divided into four groups: Group I, normal rats (Control, n = 5); Group II, diabetic rats (Diabetes, n = 6); Group III, V5dipic-NH2-treated diabetic rats (V5dipic-NH2, n = 6); Group IV, VOSO4-treated diabetic rats (VOSO4, n = 5). The VOSO4-treated group was considered as the positive control group.

Body weight and drinking water were monitored daily during the entire experiment. Blood glucose as well as diet consumption in each group were checked every four days. Blood samples were obtained from the tail vein of the rats and blood glucose levels were determined with an Accu-Chek blood glucose monitor.

2.4. Oral glucose tolerance test (OGTT)

After treatment with vanadium compounds, an oral glucose tolerance test was carried out. The rats were fasted for 12 h, and a glucose (200 mg/mL) was given by oral gavage at a dose of 2 g/kg body weight. Blood samples were obtained from the tail vein at 0, 30, 60, 90, and 180 min after glucose administration, respectively. Blood glucose levels were measured with the commercial glucose kit.

2.5. Serum biochemical parameters test

Blood samples were collected from the abdominal vein with a microsyringe. Serum was separated at 3000 rpm for 15 min. Biochemical parameters in serum, including TCHO, TG, CR, BUN, AST and ALP, were determined using an OLYMPUS AU4000 chemistry analyzer.

2.6. Statistical analysis

All data were expressed as the mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test for the multiple comparisons among the groups. Values for P < 0.05 were considered statistically significant.

3. Results

3.1. General parameters

In this study, the body weights of diabetic and V5dipic-NH2-treated diabetic rats were lower as compared with those of normal control rats throughout the experimental period of 40 days (Fig. 2A). The V5dipic-NH2-treated diabetic rats did not gain any weight over 40 days, nor did the positive control group treated with VOSO4. At the end of the experiment, the body weights in both vanadium-treated groups were slight lower (about 14%) than those in the diabetes group. The intake of elemental vanadium was calculated from the rats’ water consumption, which contained a targeted concentration of the vanadium compounds. The vanadium intake from the drinking water in the V5dipic-NH2 group was markedly lower than that in the VOSO4-group (Fig. 2B). As shown in Fig 2C and D, the diabetes group showed a significant increase in food and water consumption (P < 0.01). Oral administration of V5dipic-NH2 and VOSO4 resulted in a significant decrease in food and fluid consumption, especially at the concentration of 0.3 mg/mL.

3.2. Blood Glucose levels and OGTT

The changes of blood glucose levels are shown in Fig. 3A. The initial blood glucose levels in the diabetic and vanadium-treated rats were higher than those in the control rats. There was little if any reduction in blood glucose levels following the treatment with 0.1 mg/mL of V5dipic-NH2 and VOSO4. However, a statistically significantly decline in the blood glucose was observed in the vanadium-treated diabetic rats when the concentration of V5dipic-NH2 and VOSO4 were elevated to 0.3 mg/mL.

As shown in Fig. 3B, the blood glucose concentration of the rats rose greatly after loading with d-glucose, and then it was reduced smoothly. During the OGTT, the blood glucose of normal rats remained at less than 7 mM, while the blood glucose level in the diabetes group was consistently higher than that of the vanadium-treated groups. At the end of this test, the blood glucose level of the diabetic rats did not return to the basal levels, however glucose concentrations in both vanadium-treated groups were significantly lower than that in the diabetes group.
3.3. Serum parameters

The levels of TCHO, TG, ALP, AST, BUN and CR in serum are summarized in Table 1. Serum TCHO and TG levels in the diabetes group were higher than those in normal rats, which were significantly decreased after treatment with V5dipic-NH2 and VOSO4. Moreover, the activities of serum ALP and AST were obviously increased in diabetic rats. However, the ALP and AST activities were significantly declined in both V5dipic-NH2 and VOSO4-treated diabetic rats. The CR levels were not statistically different among the four groups of rats. The concentration of BUN in the diabetes group was more than two-fold higher compared to the control group and remained unchanged after treatment with V5dipic-NH2 and VOSO4.

4. Discussion

Insulin-mimetic effects of vanadium compounds have been widely reported in both type 1 and type 2 diabetic animal models, such as STZ or alloxan-induced type 1 diabetic rats and ob/ob, db/db, KKAy type 2 diabetic mice [24–26]. The present study focused on determining the anti-diabetic effects of V5dipic-NH2 in STZ-induced diabetic rats using VOSO4 as the positive control. In the present study, STZ-induced diabetes is characterized by a severe loss of body weight, hyperphagia and polydipsia, which is consist with previous studies [27,28]. Oral administration of V5dipic-NH2 to diabetic rats markedly decreased the food and water intake. This is consistent with the diabetes syndromes, such as hyperphagia and polydipsia, which were ameliorated by V5dipic-NH2 treatment. In addition, we found a slight decrease in body weight of V5dipic-NH2-treated rats as compared to diabetic rats, which could be a result of the ability of V5dipic-NH2 to reduce the food intake. Alternatively, this effect could also be attributed to the observed aversion of rats towards the taste of vanadium compounds [19,27], which results in reduced drink and food uptake of these animals. The blood glucose-lowering effects of both V5dipic-NH2 and VOSO4 were observed upon oral administration of the vanadium compounds to the rats at a concentration of 0.3 mg/mL of the respective vanadium compound. Furthermore, the results of the OGTT indicated that impaired glucose tolerance was improved after treatment with vanadium compounds.

Studies have shown that inhibition of protein tyrosine phosphatase 1B (PTP1B) stimulates the insulin signaling pathway in vivo [29] and in vitro [30,31]. The insulin-enhancing agents are generally known to act downstream of the insulin receptor resulting in increased insulin signaling [32,33]. That PTP1B is inhibited by vanadium compounds is well-known, and studies have shown that some vanadium compounds can be more potent than the simple salt [20]. According to the previous studies, we speculated that the anti-diabetic mechanism of V5dipic-NH2 may be explained by the inhibition of PTP1B.

Inorganic vanadium salts are also known to have an effect on the decline of hyperglycemia in diabetics [34]. However, side effects, such as gastrointestinal discomfort, diarrhea, and hepatic or
been synthesized and documented to have insulin-enhancing properties. This observation can be interpreted as V5dipic-NH₂ being more efficient at lowering hyperglycemia than VOSO₄. Moreover, diarrhea as a major side effect of vanadium compounds was not observed in the V5dipic-NH₂-treated diabetic rats.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>TCHO (mM)</th>
<th>TG (mM)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>CR (mM)</th>
<th>BUN (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.44 ± 0.28</td>
<td>0.67 ± 0.21</td>
<td>216 ± 33</td>
<td>63 ± 7</td>
<td>56 ± 5</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.04 ± 0.42</td>
<td>0.94 ± 0.09</td>
<td>257 ± 133</td>
<td>747 ± 326</td>
<td>47 ± 12</td>
<td>12.6 ± 3.0</td>
</tr>
<tr>
<td>V5dipic-NH₂</td>
<td>1.10 ± 0.22</td>
<td>0.47 ± 0.28</td>
<td>166 ± 27</td>
<td>223 ± 114</td>
<td>46 ± 5</td>
<td>12.4 ± 3.6</td>
</tr>
<tr>
<td>VOSO₄</td>
<td>0.98 ± 0.15</td>
<td>0.33 ± 0.06</td>
<td>140 ± 42</td>
<td>243 ± 59</td>
<td>45 ± 10</td>
<td>14.3 ± 5.8</td>
</tr>
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</table>

Values are expressed as mean ± SD. *P < 0.05, **P < 0.01 vs. diabetic rats.

It has been well documented that diabetics are predisposed to diabetic nephropathy and ensuing renal failure. Hyperglycemia-induced oxidative stress, activation of protein kinase C, and advanced glycation end products are the major contributors to the development of nephropathy. The STZ-induced diabetic rats developed symptoms of renal dysfunction, as shown by elevated BUN concentrations (Table 1). Serum CR and BUN were analyzed to assess the effects of V5dipic-NH₂ on kidney function in type 1 diabetic rats. A slightly higher BUN concentration was observed in the VOSO₄ treatment group compared to the diabetes group. However, V5dipic-NH₂ treatment did not change serum levels of BUN and CR, which suggested that V5dipic-NH₂ has no side effects on the kidneys of diabetic rats.

Type 1 diabetics often have dyslipidemia and altered metabolism of triglyceride-rich lipoproteins. In addition, low synthesis and high absorption of cholesterol have been observed in STZ-induced diabetic rats. Vanadium compounds have been shown to reduce the cholesterol biosynthesis though decreasing the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. Our results suggested that V5dipic-NH₂ treatment significantly reduced the elevated serum TG and TCHO, indicating a lower risk for heart disease. A previous study with KKAY mice given vanadate in water at a concentration of 100 μg/mL showed that vanadate administration caused lower fasting blood glucose and triglycerides levels similar to the results of the current study.

The insulin-enhancing properties of organic vanadium complexes have previously been compared with those of inorganic vanadium salts. One report showed that V-maltol complexes lowered the high blood glucose and hepatic glycogenesis more potently than VOSO₄. Furthermore, the fine-tuning of the vanadium with organic ligands may decline the gastrointestinal adverse effects of vanadium, possibly by elevating its bioavailability. The anti-diabetic effects of V-dipic complexes with different oxidation states on type 1 diabetic rats were compared as a function of the vanadium’s oxidation state.
whereas in this work we modified the ligand. The results indicated that the redox processes may play an important role for biological action of vanadium. Among V3dipic, V4dipic, V5dipic and VOSO4, V5dipic was most effective at lowering the blood glucose levels of STZ-induced diabetes rats [19]. In the present study, V5dipic-NH2 and VOSO4 had similar hypoglycemic effects; however, the vanadium intake in V5dipic-NH2 group was significantly lower than that in VOSO4 group. These results indicated that the anti-diabetic effects of V5dipic-NH2 V(V) is more effective than that of VOSO4 V(IV), which is consistent with the previous report [19].

In conclusion, our results suggested that V5dipic-NH2 is an effective organic vanadium compound in lowering blood glucose levels as well in improving oral glucose tolerance and lipid metabolism. Moreover, no apparent side effects were observed in the V5dipic-NH2-treated group during the whole experimental period. In addition, the compound was also found to have a protective effect on the liver dysfunction induced by hyperglycemia.

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References