

Evidence for the Improvement of Noninsulin-Dependent Diabetes Mellitus in KKA^y Mice with Daily Oral Administration of Bis(6-methylpicolinato)oxovanadium(IV) Complex

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A vanadyl complex, bis(6-methylpicolinato)oxovanadium(IV), VO(6MPA), with VO(N₂O₂) coordination mode, was found to exhibit a normoglycemic effect on KKA^y mice with hereditary noninsulin-dependent diabetes mellitus with daily oral administration.

Key words vanadyl complex, insulinomimetic activity, oral administration, VO(N₂O₂) coordination, NIDDM

Recent intensive research has demonstrated that exogenous vanadyl (oxidation state +4) and vanadate (oxidation state +5) ions both mimic the biological actions of insulin in rat adipocytes in terms of hexose uptake, stimulation of glucose oxidation, and inhibition of lipolysis.^{1–4} However, due to the low insulinomimetic activity of vanadyl (VOSO₄) attributed to the solubility and stability of vanadyl ions at neutral pH values, and permeation into cell interior, vanadate ions were exclusively used in such experiments.^{6,7} Vanadate ions administered orally (in drinking water) to streptozotocin (STZ)-induced hyperglycemic rats, a model of insulin-dependent diabetes mellitus (IDDM), have been demonstrated to reduce the high levels of circulating glucose levels to normal values.^{8,9} Moreover, it was found that vanadate administration is also effective in experimental models of noninsulin-dependent diabetes mellitus (NIDDM). Oral vanadate therapy in *db/db* and *ob/ob* mice induced normoglycemia,¹⁰

while oral administration of insulin failed to do so.¹¹

Attempts to use vanadyl ions have been made, because vanadate ions have the disadvantage of being 6- to 10-fold more toxic than vanadyl ions.¹² To overcome the solubility of vanadyl ions, autoxidation of vanadyl ions to vanadate, and vanadium toxicity in animals, we have used vanadyl complexes with different coordination modes involving VO(O₄), VO(N₂O₂), VO(N₂S₂), and VO(S₄).^{5,13} In those systemic investigations, we first examined the complexes for the *in vitro* insulinomimetic activities such as potentiation of glucose incorporation in rat adipocytes and suppression of free fatty acid (FFA) release from adipocytes treated with epinephrine,^{14,15} and then evaluated the *in vivo* insulinomimetic action in STZ-induced hyperglycemic rats (STZ rats) with IDDM by intraperitoneal (i.p.) injection or oral administration.^{14–18} Among the complexes, we found that bis(6-methylpicolinato)oxovanadium(IV) (VO(6MPA)) complex is the most effective agent in treating the hyperglycemia of STZ rats in intraperitoneal injection as well as oral administration.¹⁸ On the basis of these observations and results, we further attempted to use VO(6MPA) to treat hyperglycemic animals with NIDDM.

To the authors' knowledge, this paper reports the first example of treating hyperglycemic KKA^y mice with NIDDM with the vanadyl complex VO(6MPA) given daily by oral administration.

VO(6MPA) was prepared in aqueous solution, pH 6–7, by mixing 6-methylpicolinic acid (Tokyo Kasei Organic Chemical, Tokyo, Japan) and VOSO₄ (VS) (Nakalai Tesque, Kyoto, Japan) in a molar ratio of 2 : 1, respectively, and washed with water, as reported previously.¹⁸

KKA^y mice (Clea Japan, Tokyo, Japan) weighing 30–35 g were divided into 4 groups: 1) control group; 2) intraperitoneal injection VS-treated group; 3) intraperitoneal injection VO(6MPA)-treated group; and 4) oral VO(6MPA)-treated

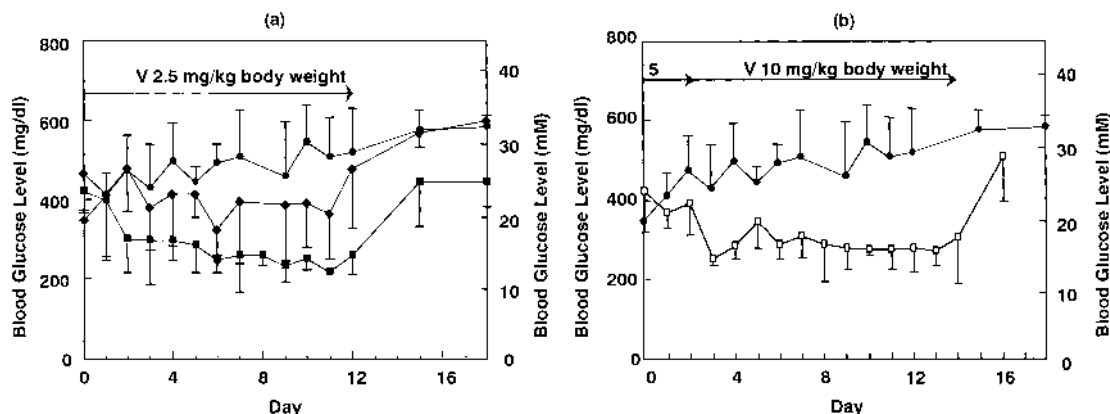
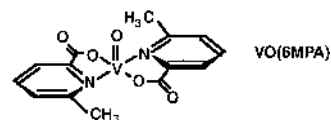


Fig. 1. Changes in Blood Glucose Level in KKA^y Mice Given VOSO₄ or VO(6MPA) by Daily Injection (Left) (0.049 mmol/kg Weight i.p. for 12 d and Then No Treatment) and Oral Administration (Right) (0.098 mmol/kg Body Weight for the First 2 d and 0.196 mmol/kg for the Following 12 d and Then No Treatment)

Each symbol represents the mean ± S.D. for five mice. —●— control mice, —■— VO(6MPA)-treated mice by intraperitoneal injection, —◆— VOSO₄-treated mice by intraperitoneal injection, and —□— VO(6MPA)-treated mice by oral administration.

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Table 1. Serum parameters of KKA^y Mice Treated with VO(6MPA) by Oral Administration for 14 d

Treatment	Glucose (mg/dl)	Insulin (mU/ml)	FFA (mEq/l)	BUN (mg/dl)
None	578±50	310±35	0.89±0.15	32.4±6.5
VO(6MPA)	273±39*	236±39*	1.04±0.19	28.5±4.5

VO(6MPA) was given by daily oral administration to KKA^y mice at doses of V 0.098 mmol/kg body weight for first 2 d and V 0.196 mmol/kg for the following 12 days. Data are expressed as the mean±S.D. for five mice. * Significance: $p < 0.01$ vs. no treatment (Student's *t*-test).

group. VO(6MPA) was suspended in 5% acacia and VS was solved in saline. Mice received daily injections of VS or VO(6MPA) at a dose of vanadium (V) 2.5 mg/kg body weight (0.049 mmol/kg) i.p. for 12 d or daily oral administration at doses of V 5 mg/kg (0.098 mmol/kg) for the first 2 d and then V 10 mg/kg (0.196 mmol/kg) for the following 12 d at about 11:00 after weighing and determination of their serum glucose levels. On days 0 and 14 of the experiments, serum parameters, such as insulin (Insulin-EIA Wako kit), FFA (NEFA C Wako kit), and blood urea nitrogen (BUN) (Fuji Dry-Chem 3000 V) of KKA^y mice in the oral administration group were measured.

When VO(6MPA) on VS was given to KKA^y mice at the dose of V 2.5 mg/kg i.p. for 12 d, blood glucose levels decreased within 4 or 5 d and the levels were maintained during the administration period, while the blood glucose level of the control group was hyperglycemic (Fig. 1(a)). The normoglycemic action of VO(6MPA) was stronger than that of VS. The body weight of KKA^y mice in each group increased gradually, and the body weight change between groups was not significant.¹⁹⁾ The blood glucose level of KKA^y mice treated with oral VO(6MPA) V 5 mg/kg for 2 d and then V 10 mg/kg for 12 d decreased within 4 d and the decrease was maintained during the administration period (Fig. 1(b)) as the body weight increased.²⁰⁾ After cessation of complex administration, serum glucose levels increased gradually in mice in both administration groups (Fig. 1). This differed from the results in STZ rats, in which normoglycemia was maintained after cessation of administration.¹⁸⁾ The fluid intake and food consumption in the VS- and VO(6MPA)-treated intraperitoneal injection groups as well as in the oral administration group were reduced by about 20% compared with the control group. However, no body weight loss was observed.^{19,20)} The serum parameters of KKA^y mice treated with VO(6MPA) by oral administration for 14 d (Table 1) suggested that high blood glucose, BUN levels, and hyperinsulinemia of KKA^y mice were improved by complex administration. On the basis of these results, the vanadyl complex VO(6MPA) improved NIDDM without body weight loss.

The experimental advantage of using vanadyl compounds to treat diabetes mellitus has been shown in terms of vanadyl incorporation into adipocytes¹⁵⁾ and the presence of vanadyl-dependent pathways such as membrane protein tyrosine phosphatases (PTPases) and cytosolic protein tyrosine kinases.^{1-3,21-23)} The PTPases are inhibited *in vitro* and *in vivo* by vanadium compounds.²⁴⁻²⁶⁾ Thus vanadium compounds enhance insulin receptor and/or substrate phosphorylation indirectly by inhibiting the dephosphorylation of these proteins, which in turn induce normoglycemia in diabetic animals.

The present study indicates that VO(6MPA) has a normoglycemic effect not only in IDDM model rats¹⁸⁾ but also in NIDDM model mice. Moreover, the high glucose levels as well as high BUN levels observed in NIDDM mice were improved. Therefore VO(6MPA) is expected to be effective in treating milder forms of NIDDM such as that in humans. Furthermore, it has recently been reported that oral vanadate and vanadyl ions both improve the insulin resistance and glucose tolerance that are characteristic of NIDDM patients.^{22,23)} Thus the lipophilic vanadyl complex VO(6MPA)¹⁸⁾ is a more potent oral therapeutic agent in treating both IDDM and NIDDM than vanadium ions. A more refined investigation involving the mechanism of action of VO(6MPA) is in progress.

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- 19) The body weights of the mice were measured daily during administration of the compounds. On days 0 and 12 of the experiments, the body weights of the control mice and those treated with intraperitoneal injection of VS and VO(6MPA) were 36.7±1.8 g and 42.7±2.4 g, 34.8±1.2 g and 44.1±2.0 g, and 36.4±2.1 g and 44.2±2.9 g, respectively.
- 20) On days 0 and 14 of the experiments, the body weights of the mice treated with oral VO(6MPA) were 35.0±1.1 g and 37.4±0.9 g, respectively.
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