Insulin-Mimetic Action of Vanadyl Complexes

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Summary Blood glucose levels of streptozocin (STZ)-induced diabetic rats dropped from hyperglycemic levels to hypoglycemic levels within 24–48 h after treatment with vanadyl sulfate (VS) by intraperitoneal injection. Results of the glucose tolerance test indicated that the diabetes was completely improved by VS administration, but the plasma insulin levels were still low. Determination of both vanadyl and total vanadium in VS-treated STZ-rats suggested that the vanadyl is possibly in a pharmacologically active form. Several vanadyl complexes such as vanadyl-cysteine methyl ester (VCys), -oxalate (VOX), -malonate (VMA), -salicylaldehyde (VSA), and -(+)-tartarate (VTA) were tested by oral administration. The order of normoglycemic effect in STZ-rats was VMA > VCys > VTA > VSA > VOX. The action of VCys was dose-dependent in the range of 1–10 mg V/kg body weight, and this complex was shown to be a potent agent in restoring the normoglycemic level in STZ-induced diabetic rats.

Key Words: insulin-like action, vanadyl complexes, diabetes, vanadium determination, electron spin resonance

Vanadium is an essential trace element of unknown function(s) and an endogenous constituent of almost all mammalian organs [1–4]. Recently, vanadium was found at the active site of a nitrogenase from the Gram-negative bacterium *Azotobacter chroococcum* [5] and of a bromoperoxidase from the marine algae *Ascophyllum nodosum* [6]. Two vanadium-containing natural

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products amavadin [7] and vanoxonin [8] have been isolated from fungi. Furthermore, since 1911, certain species of marine organisms called ascidians have been known to accumulate vanadium in their blood from sea water [9–11], yet the physiological functions and chemical structures of vanadium in the animals are not clear [9–11]. On the other hand, the vanadate ion (+5 oxidation state of vanadium) was found to be a potent inhibitor of Na^+ , K^+ -ATPase in 1977 [12]; and as a result, physiological, biological and bioinorganic chemical interests in the biological functions of vanadium have been focused on the structures and mechanism of actions of this element in living organisms. In the 1980's, the vanadate ion was shown to mimic the action of insulin in intact cell systems [13–18]. Heyliger *et al.* in 1985 [19], followed by Meyerovitch *et al.* in 1987 [20] and Blondel *et al.* [21] found that the inclusion of sodium vanadate in the drinking water of streptozocin (STZ) [22]-induced diabetic rats alleviated some symptoms of diabetes, resulting in the normalization of blood glucose levels. In these studies, sodium vanadate was exclusively used. However, sodium vanadate is reported to be more toxic to rats than vanadyl sulfate (+4 oxidation state).^{*} We recently found that the distributions of vanadyl species and total vanadium in organs of rats treated with vanadyl sulfate or sodium vanadate are not different from each other [24]. Thus, we studied whether the less toxic vanadyl sulfate and various types of vanadyl complexes (Fig. 1) can normalize the blood glucose levels in STZ-induced diabetic rats.

In this paper we report the first experiment, in which both vanadyl sulfate (VS) and vanadyl-cysteine methyl ester complex (VCys) [25, 26] are shown to have an excellent normalization effect on blood glucose levels in STZ-induced diabetic rats (STZ-rats), an effect comparable to that of sodium vanadate.

MATERIALS AND METHODS

VCys was prepared as described previously [25, 26]. Other vanadyl complexes (Fig. 1) were synthesized in our laboratories with modification of the methods described previously [27] for (II), [28] for (III), [29] for (IV) and (V). Diabetes was induced in male Wistar rats (190–200 g body weight) by a single intravenous injection of a freshly prepared solution of STZ (60 mg/kg body weight) in 0.1 M citrate buffer, pH 5.0. The STZ-rats were given a saline solution of VS (50 mg $\text{VO SO}_4 \cdot 6\text{H}_2\text{O}$ /kg body weight for the first two days and then 25 mg/kg body weight) daily by intraperitoneal (i.p.) injection of suspensions of vanadyl complexes in 5% acacia (for example, 6.6–65.8 mg $\text{VO}(\text{CysMe})_2$ /kg body weight or 1–10 mg V/kg body weight) daily by oral administration. Blood glucose levels were measured by the *o*-toluidine method [30]. Glucose tolerance test was performed at a dose of 3 g glucose/kg body weight, and plasma insulin levels were estimated with an Insulin Kit Wako B (Wako Pure Chemical Industries, Ltd., Osaka).

^{*}Lethal doses of vanadyl sulfate and sodium vanadate are reported to be 158–190 mg/kg body weight and 10–20 mg/kg body weight, respectively, to rats [23].

Characterization of vanadyl species [31, 32] and concentrations of vanadyl and total vanadium in rat organs were evaluated by electron spin resonance (ESR) spectrometry, with modification of the method reported previously [33]. ESR spectra were recorded in a JES-FEIXG ESR spectrometer (JEOL Ltd., Tokyo) at 77K, modulation frequency 100 kHz, modulation amplitude 6.3 G, microwave power 5 mW. Magnetic field was calibrated with a frequency counter, TR 5212 (Takeda Riken Co., Ltd., Tokyo). As standards, tetracyanoquinodimethane-lithium salt (TCNQ-Li) ($g=2.00252$) and Mn(II) in MgO ($\Delta H_{3-4}=86.9$ G) were used.

RESULTS AND DISCUSSION

As shown in Fig. 2, blood glucose levels of STZ-rats dropped from hyperglycemic levels to hypoglycemic levels within 24–48 h after treatment with VS given by i.p. injection. This observation is very analogous to that of the effect with sodium vanadate given orally in the drinking water [19–21]. When sodium vanadate was administered at a dose of 30 mg NaVO_3/kg body weight by i.p. injection, the STZ-rats died within 10 h. The glucose tolerance test for VS-treated STZ-rats gave a time-dependent curve (maximum 200 mg glucose/dl plasma), similar to that of healthy rats, while untreated STZ-rats showed high blood glucose levels, maximally 600 mg glucose/dl plasma (data not shown). However, the insulin levels were not improved by VS administration in STZ-rats (insulin levels: control $25.6 \pm 1.6 \mu\text{U/ml}$, STZ-rats $2.8 \pm 0.5 \mu\text{U/ml}$ and VS-treated STZ-rats $4.9 \pm 5.6 \mu\text{U/ml}$). To get an insight into the chemical forms of vanadium in organs of VS-treated STZ-rats, we measured ESR spectra at 77K (Fig. 3). Consisting of an eight-line signal, ESR spectra due to VO^{2+} were clearly observed in the liver, kidney, and serum. Comparison of these ESR parameters ($g_{\parallel}$ - and

Fig. 1. Structures of vanadyl-cysteine methyl ester(VCys, I), -oxalate(VOX, II), -salicylaldehyde(VSA, III), -malonate(VMA, IV), and -(+)-tartarate(VTA, V) complexes.

Fig. 2. Basal blood glucose changes in STZ-diabetic rats treated with VS (—○—, 50 mg/kg for the first two days and then 25 mg/kg body weight by i.p. injection) or VCys (—●—, 65.8 mg/kg body weight by oral administration). Diabetic rats were given these vanadium compounds 7–9 days daily after the induction of diabetes by STZ (60 mg/kg). Administration of the complexes was started at 0 day in the figure. Each point is the mean $\pm$ SD of 3 and 10 rats in each group receiving VS and VCys, respectively.

Fig. 3. ESR spectra (77K) of fresh organs of STZ-rats treated with VS. (A) Liver, (B) kidney, and (C) serum. Amplitude for recording was 125, 25, and 500 for (A), (B), and (C), respectively.

$A_{//}$ -values) with those of several vanadyl complexes (Table 1) indicates that the vanadyl ion in organs binds to ligands such as the oxygen of water or of oxo-amino acid residues of proteins, which is similar to the results on healthy animals [34]. Quantification of both vanadyl and total vanadium levels in VS-treated STZ-rats by ESR spectrometry showed that over 90% of total vanadium was present in the vanadyl form (Fig. 4). Because of its hypoglycemic action in animals, the vanadium in organs is probably in a pharmacologically active form.

On the basis of these results, the effectiveness of VS to improve the experimen-

Table 1. ESR parameters of vanadyl species in organs of STZ-rats treated with VS and of several model vanadyl complexes.^a

Organs or ligands	Coordination mode	ESR parameter	
		$g_{ }$	$A_{ }$ ($\times 10^{-4} \text{ cm}^{-1}$)
Liver (A)		1.949	185
Kidney (B)		1.947	184
Serum (C)		1.940	184
Water	O_4	1.934	183
OX (pH=1)	O_4	1.938	174
DL-Serine (pH=11)	N_2O_2	1.947	165
Cys (pH=10)	S_2N_2	1.964	144
2-Mercaptoethanol (pH=10)	S_2O_2	1.970	145

^a See references [31, 32]. Fig. 4. Organ distribution of total vanadium and vanadyl form in STZ-induced diabetic rats treated with VS for one month. Total vanadium and vanadyl form were determined by ESR spectrometry [18, 19]. Data are expressed as the mean $\pm$ SD of 3 rats. ■, Total vanadium; □, vanadyl form.

tal diabetes was clearly established, and thus we tested the influence of orally administered vanadyl complexes such as vanadyl-cysteine methyl ester (VCys), -oxalate (VOX), -malonate (VMA), -salicylaldehyde (VSA), and -(+)-tartarate on the blood glucose levels in STZ-rats. Among them, we found that VMA and VCys given by oral administration were the most effective (Table 2 and Fig. 2) in giving the normoglycemic levels, the order of the blood glucose lowering effect being $\text{VMA} > \text{VCys} > \text{VTA} > \text{VSA} > \text{VOX}$ at the dose of 10 mg V/kg body weight.

When the vanadyl complexes were administered, the level of vanadium in the

Table 2. Relationship between vanadyl complex and the hypoglycemic effect in STZ-induced diabetic rats 24 h after administration of the complex.

Complex	Dose of vanadium administered (mg V/kg)	Blood glucose level (mg/dl)	
Control		412±61	(100)
VCys	3	332±91	(81)
VCys	10	254±165	(62)
Control		478±40	(100)
VOX	3	468±45	(98)
VOX	10	385±83	(81)
VMA	3	367±132	(77)
VMA	10	251±132	(53)
Control		376±70	(100)
VSA	3	337±116	(90)
VSA	10	275±107	(73)
VTA	3	367±132	(98)
VTA	10	254±132	(68)

Vanadyl complexes were given by oral administration, and the blood glucose levels were measured after 24 h. The value in parenthesis is the relative blood glucose level when the level of control diabetic rats is taken as 100%.

Fig. 5. Dose-dependent hypoglycemic effect of VCys complex given by oral administration. (---△---), Control diabetic rats; (—●—), VCys 1 mg V/kg; (—□—), VCys 3 mg V/kg; (—○—), VCys 10 mg V/kg.

blood was found to be the highest in the rats treated with VCys (data not shown). Thus we examined the effect of VCys more closely. The blood glucose-lowering effect of the VCys complex was observed to be dose-dependent (6.6–65.8 mg/kg) (Fig. 5) and was clearly demonstrated at the dose of 10 mg V/kg body weight. Furthermore, all animals that received 10 mg V/kg body weight of VCys showed no apparent signs of toxicity. The toxicological study of VCys was performed in rats by oral administration for 4 weeks. All animals (6/6) receiving 100 mg/kg of VCys died due to diarrhea within four days. In all animals receiving dosages of up to 65.8 mg/kg, no significant changes in body weight, food intake, general symptoms, organ weights, or necropsy were observed. After the fasting prior to sacrifice, diarrhea was observed in all of these animals, although it was not noted during the

course of the studies. A slight increase in GPT activity and a decrease in ALP activity were observed in the rat plasma at the dose of 65.8 mg/kg. These changes were not significant and were supposedly caused by diarrhea rather than by the direct toxic action of VCys. A slight exofoliation of the intestinal mucous membrane was noted in the histopathological study on the animals receiving 65.8 mg/kg of VCys. Thus the VCys complex is a good compound for studying the mechanism of the normalization of blood glucose levels in STZ-rats.

In conclusion, this study shows that (1) vanadyl complexes such as VCys can normalize the blood glucose levels in STZ-rats, similarly to vanadate; and (2) VCys shows no significant toxicity when it is given by oral administration, thus suggesting its possible use clinically to treat diabetes. Further investigation is obviously required before any clinical application of VCys could be made.

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