A New Concept: The Use of Vanadium Complexes in the Treatment of Diabetes Mellitus

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ABSTRACT: In the 21st century, patients suffering from diabetes mellitus (DM), a lifestyle-related disease, will increase more than in the 20th century. DM is threatening because of the development of many severe secondary complications, including atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormalities, diabetic retinopathy, and ocular disorders. Generally, DM is classified as either insulin-dependent type 1 or noninsulin-dependent type 2 DM. Type 1 DM is treated only by daily insulin injections; type 2 DM is treated by several types of synthetic therapeutic substances together with a controlled diet and physical exercise. Even with these measures, the daily necessity for several insulin injections can be painful both physically and mentally, whereas the synthetic therapeutic substances used over the long term often have side effects. For those reasons, the creation and development of a new class of pharmaceuticals for treatment of DM in the 21st century would be extremely desirable. In the last half of the 20th century, investigations of the relationships among diseases and micronutrients, such as iron, copper, zinc, and selenium, have been numerous. Research into the development of metallopharmaceuticals involving the platinum-containing anticancer drug, cisplatin, and the gold-containing rheumatoid arthritis drug, auranofin, has also been widespread. Such important findings prompted us to develop therapeutic reagents based on a new concept to replace either insulin injections or the use of synthetic drugs. After many trials, we noticed that vanadium might be very useful in the treatment of DM. Before the discovery of insulin by Banting and Best in 1921 and its clinical trial for treating DM, the findings in 1899, in which orally administered sodium vanadate (NaVO₃) was reported to improve human DM, gave us the idea to use vanadium to treat DM. However, it has taken a long time to obtain a scientific explanation as to why the metal ion exhibits insulin-mimetic or blood-glucose lowering effects in vitro and in vivo experiments. After investigations from many perspectives involving biochemistry and bioinorganic chemistry, vanadyl sulfate (VOSO₄) and its complexes with several types of ligands have been proposed as useful for treating DM in experimental diabetic animals. On the basis of a mechanistic study, this article reports on recent progress regarding the development of antidiabetic vanadyl complexes, emphasizing that the vanadyl ion and its complexes are effective not only in treating or relieving both types of DM but also in preventing the onset of DM.


Introduction

Diabetes mellitus (DM), a lifestyle-related disease and one of the most widespread diseases of our times, is important because DM develops many severe secondary complications, including...
atherosclerosis (a disease resulting in loss of elasticity from destruction of the elastic fibers in the blood vessels), microangiopathy (disorders of the blood capillary), renal (kidney) dysfunction and failure, cardiac (heart) abnormalities, diabetic retinopathy (functional defect of the retina, which relates to the whole body disorder), and ocular disorders (eye disorders that often induce blindness). Generally, DM is classified as either insulin-dependent type 1 DM (caused by destruction of pancreatic B cells) or noninsulin-dependent type 2 DM (caused by aging, obesity, spiritual stress, or other environmental factors), which are treated by daily injections of insulin or several types of synthetic therapeutic substances, respectively. Unfortunately, these methods of treatment have some defects: the injections of insulin several times a day are painful and elevate the levels of patient stress, especially in young people, and synthetic therapeutic substances often have some side effects. For these reasons, the creation and development of new therapeutic substances to replace insulin injections and synthetic drugs during the 21st century are extremely desirable.

Numerous factors, such as genetics, environment, eating habits, physiological state, hormones, and stress are considered to be associated with the development of DM. Before Banting and Best’s discovery of insulin in 1921 and its clinical trial for treating DM, an interesting result was reported in 1899 in which orally administered sodium vanadate (NaVO₃) was found to be effective in improving the conditions of patients with DM. Because of the finding in 1899 and an increased interest in the nutritive values of trace elements in the latter half of this past century, the correlation between DM and trace elements has been studied with a focus on the micronutrient status of patients with DM and the therapeutic effects of trace elements.

Many studies have revealed changes in the status of trace elements in patients with DM. Deficiencies in certain trace elements have been found to correlate with the development of DM as well as the presence of diabetic complications. However, the most interesting finding has been the treatment or improvement of symptoms of both types of DM by metal ions and their complexes. Since 1899, several metal ions, including vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), zinc (Zn), selenium (Se), molybdenum (Mo), and tungsten (W), and their complexes have been reported as showing insulin-mimetic activity in in vitro experiments and to exert positive treatment effects on experimental animals and subjects with DM. The effective chemical forms of the metal ions and complexes causing antidiabetic activities in experimental animals and subjects with DM are summarized in Table 1.

In the last half of the 20th century, several clinically useful metallopharmaceuticals such as the platinum-containing anticancer drug, cisplatin, the gold-containing antirheumatoid arthritis drug, auranofin, and the aluminum- and zinc-containing antiulcer drugs, scarlate and polaplezinc, respectively, were developed. Following the development of these important metallopharmaceuticals, metal-containing antidiabetic agents backed by new concepts are expected. In the
Diabetes Treatment by Vanadium Complexes

Table 1. Metal ions and the complexes with antidiabetic activity in experimental animals and the subjects with DM.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Ionic form</th>
<th>Complex form</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Vanadyl sulfate (VOSO₄)</td>
<td>Bis(methyl cysteinate)oxovanadium(IV)</td>
<td>[2],[3]</td>
</tr>
<tr>
<td></td>
<td>Sodium vanadate (NaVO₃)</td>
<td>Bis(maltolato)oxovanadium(IV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bis(picolinato)oxovanadium(IV)</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>—</td>
<td>Bis(picolinato)chromium(III)</td>
<td>[4],[5]</td>
</tr>
<tr>
<td>Mn</td>
<td>Manganese chloride (MnCl₂)</td>
<td>—</td>
<td>[6]</td>
</tr>
<tr>
<td>Co</td>
<td>Cobalt chloride (CoCl₂)</td>
<td>—</td>
<td>[7]</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc chloride (ZnCl₂)</td>
<td>Bis(picolinato)zinc(II)</td>
<td>[8],[9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bis(maltolato)zinc(II)</td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>Sodium selenite (Na₃SeO₄)</td>
<td>—</td>
<td>[10]</td>
</tr>
<tr>
<td>Mo</td>
<td>Sodium molybdate (Na₃MoO₄)</td>
<td>—</td>
<td>[11]</td>
</tr>
<tr>
<td>W</td>
<td>Sodium tungstate (Na₃WO₄)</td>
<td>—</td>
<td>[12]</td>
</tr>
</tbody>
</table>

Fig. 1. A proposed mechanism of glucose incorporation and free fatty acids (FFA) release in isolated rat adipocytes.

present article, recent progress in the development of antidiabetic vanadyl (+4 oxidation state of vanadium) complexes is reviewed.

Evaluation of Insulin-Mimetic Activity of Metal Ions

The establishment of a reliable in vitro appraisal system is necessary to evaluate metal ions for their potential antidiabetic activity. For this purpose, we have developed an appraisal system with respect to the interaction of metal ions by isolating Wistar rat adipocytes (adipose cells prepared from the epididymal fat tissue) treated with adrenaline (epinephrine). The insulin action is expressed as the degree of enhancement of glucose incorporation into the cells as well as the inhibition of the release of free fatty acid (FFA) from the cells.¹⁴ The proposed system is shown in Figure 1. Although the estimate of glucose incorporation was based on the use of radioisotope-labeled glucose, the FFA release was examined with a simple determination kit that was not based on the use of radioisotopes. We used this simple and convenient FFA measuring kit
to test the efficacy of metal ions under the same experimental conditions in terms of FFA release. We termed a metal ion that was added to the system in place of insulin and which was observed to elicit FFA release from the cells in a fashion similar to the action of insulin an insulin-mimetic metal ion. Toxic metal ions, Hg²⁺, Se⁴⁺, and Cd²⁺, strongly inhibited FFA release, followed by V⁵⁺, V⁴⁺, Zn²⁺, and Mn²⁺. Essentially no insulin-mimetic activity was observed with Se⁶⁺, V⁵⁺, and W⁶⁺. V⁴⁺ is readily oxidized to V⁵⁺ (VO²⁺) and V⁶⁺ at physiological pH, but V⁴⁺ is known to be less toxic than V⁵⁺ in experimental animals (Fig. 2). Consequently, we first used V⁴⁺ (VO²⁺) in our study.

Antidiabetic Vanadyl Complexes

Although sodium vanadate (NaVO₃) was used to treat human DM in 1899,¹ we used vanadyl sulfate (VOSO₄), because it is less toxic to rats than vanadate, and most vanadium in the organs of rats administered NaVO₃ is present in the vanadyl form.²,³ However, the rates of absorption and incorporation of the inorganic ion are generally low. Accordingly, we used vanadyl complexes that were prepared with VOSO₄ and low-molecular weight ligands (Table 2).

In 1990, we first reported that bis(methylcysteinate) [VO(cysm)₂]⁻, bis(oxalato) [VO(ox)₂]⁻, bis(malonato) [VO(mal)₂]⁻, and bis(salicylaldehyde) [VO(sal)₂]⁻-oxovanadium(IV) and bis((+)-tartarato)dioxovanadium(IV) [(VO)₂(tar)₂] complexes with either the VO(S₂O₂) or VO(O₄) coordination mode showed the effects of lowering blood glucose in streptozotocin (STZ)-induced type 1 diabetic rats (STZ rats) when orally administered daily, similar to the daily administration of VOSO₄ by intraperitoneal (i.p.) injection (Fig. 3).¹⁵,¹⁶ The order of the effects of blood-glucose reduction in STZ rats was found to be VO(mal)₂ → VO(cysm)₂ → (VO)₂(tar)₂ → VO(sal)₂ → VO(ox)₂, with the action of the complexes being dose-dependent in the vanadium concentration range of 1–10 mg kg⁻¹ body weight. The trans-VO(cysm)₂ complex with a strong V=O bond,²⁷ which was analyzed by X-ray structure and found to contradict Pearson’s HSAB (hard and soft acids and basis) principle, was determined to be a good reagent for treating experimental type 1 DM. We paid close attention to the preparation of the vanadyl complexes with the V=S coordination mode, testing them with an in vitro evaluation system.

The bis(pyridinyl-N-carbodithioato)oxovanadium(IV) [VO(pcd)₂] complex was found to be the most effective among the prepared complexes with the VO(S₄)₂ coordination mode, being dose-dependent in the in vitro system in treating type 1 STZ rats by both daily i.p. injections and oral administration.¹⁸,¹⁹ In addition, the bis(1-oxy-2-pyridinethiolato) oxovanadium(IV) [VO(opt)₂] complex with the VO(S₂O₂) coordination mode demonstrated strong in vitro insulin-mimetic activity in a dose-dependent manner.²⁰,²¹ Interestingly, the VO(opt)₂ complex was effective in treating both type 1 diabetic STZ rats and type 2 obese diabetic ob/ob mice when given daily i.p. injections and oral administration.²² Tumor necrosis factor-α (TNF-α) is well known as a key factor in the obesity-diabetes link, and an elevated expression of TNF-α is observed in the epidermal and subcutaneous fat tissue of ob/ob mice. The VO(opt)₂ complex treated DM in ob/ob mice by improving the impaired glucose tolerance and attenuated the TNF-α-induced decrease in insulin receptor substrate-1 (IRS-1) phosphorylation in the adipocytes. From this, it was hypothesized that the activity of the complex was the result of attenuation of the impaired insulin signal transduction through activation of insulin receptor substrate-Pi as it related to inhibition of protein tyrosine phosphatase. As such, VO(opt)₂ is thought to have a clinical potential with regard to the treatment of obesity in type 2 DM.

Insulin-mimetic vanadyl complexes with the VO(O₄) coordination mode have been developed. Among them, bis(maltolato)oxovanadium(IV) [VO(mal)₂] has been established as effective in treating STZ rats when the complex is given by way of drinking water.²³–²⁵ The effectiveness of the bis(ethylmaltolato)oxovanadium(IV) complex has also been reported.²⁶ Other interesting candidate complexes of vanadyl, with ligands such as pyrone, pyridinone,²⁷ hydroxyazine-type heterocycles,²⁸ and amino acid related compounds,²⁹–³¹ have been prepared and their in vitro insulin-mimetic activities have been demonstrated.

Another type of vanadyl complex with the VO(N₂O₂) coordination mode, the bis(picolinato)oxovanadium(IV) [VO(pic)₂] complex, was proposed in 1995 after evaluations...
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Table 2. Insulin-mimetic vanadyl complexes with different coordination modes.

<table>
<thead>
<tr>
<th>Mode</th>
<th>Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N_2S_2)</td>
<td>(\text{VO}(\text{cysm})_2)</td>
</tr>
<tr>
<td>(S_4)</td>
<td>(\text{VO}(\text{pdc})_2)</td>
</tr>
<tr>
<td>(S_2O_2)</td>
<td>(\text{VO}(\text{opt})_2)</td>
</tr>
<tr>
<td>(N_2O_2)</td>
<td>(\text{VO}(\text{pic})_2) (\text{VO}(6\text{mpa})_2) (\text{VO}(5\text{ipa})_2)</td>
</tr>
<tr>
<td>(O_4)</td>
<td>(\text{VO}(\text{ox})_2) (\text{VO}(\text{sa})_2) (\text{VO}(\text{mal})_2) (\text{VO}(\text{ma})_2) ((\text{VO})_2(\text{tar})_2)</td>
</tr>
</tbody>
</table>

of *in vitro* insulin-mimetic activity in the adipocyte system, which was followed by a reduction in blood glucose in type 1 diabetic STZ rats, when the complex was administered daily by i.p. injections and oral administration. This complex has the advantage of having several analogues that enable examination of the structure-activity relationship of antidiabetic activity, with bis(6-methylpicolinato)oxovanadium(IV) \([\text{VO}(6\text{mpa})_2]\) and bis(5-iodopicolinato)oxovanadium(IV) \([\text{VO}(5\text{ipa})_2]\) having been found to better exhibit both *in vitro* insulin-mimetic activity and blood-glucose reduction in STZ rats. In particular, the former was found to be long acting for at least 80 days after the end of the period of oral administration. The characteristic feature of this complex might be the accumulation of vanadium in bone tissue, as determined by neutron activation analysis (NAA). However, it remains to be determined if the metal directly contributes to the manifestations of DM. In addition, the \(\text{VO}(6\text{mpa})_2\) complex has been found to reduce high blood-glucose levels of a hereditary type 2 DM animal, the KK-A' mouse, as a result of daily i.p. injections and oral administration.
In general, good-quality crystals of VO(pic)$_2$ and related complexes suitable for X-ray structure analysis were difficult or impossible to obtain. Therefore, the structures of the complexes were determined by elemental analysis and characteristic electronic, EPR, and EXAFS spectra, revealing that VO(pic)$_2$, VO(3mpa)$_2$, and VO(5ipa)$_2$ complexes have a six-coordinate structure with an additional V\(\overline{\text{O}}H_2\) bond. In contrast, VO(6mpa)$_2$ and VOSO$_4$ have no coordinate water molecule and a five-coordinate structure. We were, however, able to analyze the structure of bis(6-ethylpicolinato)oxovanadium(IV) [VO(6epa)$_2$] by X-ray (Fig. 4). Two distinct molecules were observed in an asymmetric unit. Each vanadium in VO(6epa)$_2$ was coordinated by two carboxylate oxygens, two pyridine nitrogens, one vanadyl oxygen, and one water oxygen, producing a distorted octahedral geometry. The two carboxylate oxygens and the pyridine nitrogens occupied an equatorial plane; the two ligands coordinated the vanadium in a trans arrangement.

Chemical Speciation, Metallokinetic Analysis and Gastrointestinal Absorption of the Vanadyl State

The clinical use of vanadium ions such as VOSO$_4$ and NaVO$_3$ as well as vanadyl complexes is anticipated. For this reason, it is essential to understand the organ distribution of vanadium, the chemical speciation of vanadium in tissues, and the metallokinetic features of vanadium ions as well as vanadium complexes. Organ and subcellular distributions of vanadium after administration of VOSO$_4$ and its complexes were examined by neutron activation analysis (NAA). In rats given VOSO$_4$, vanadium was found in the kidney \(\rightarrow\) liver \(\rightarrow\) bone \(\rightarrow\) pancreas; whereas in rats treated with complexes like VO(5ipa)$_2$, the metal was determined in bone \(\rightarrow\) kidney \(\rightarrow\) spleen \(\rightarrow\) liver \(\rightarrow\) pancreas. The differences in distribution between ionic and complex forms of the vanadyl species could possibly account for the differences in lipophilicity and toxicity as well as for the long-lasting characteristics of the complexes. This is another area where further study is needed.

Chemical speciations of vanadyl species have been revealed in artificial serum system and in specific organs of rats, as estimated by potentiometric titration and electron spin echo envelope modulation (ESEEM) methods. The formation of bisligand complexes as well as of ternary complexes consisting of a ligand, vanadyl, and low molecular weight serum components has been suggested as being responsible for the insulin-mimetic activity of the administered complexes. ESEEM results for the liver and kidney of rats treated with VO(pic)$_2$ indicate that some picolinate species, including both the bispicolinate complex and a
partially decomposed picolinate-vanadyl-protein or amino acid complex, exists in the treated organs.  

The metallokinetic features of the vanadyl species in the blood of rats given VOSO₄ and the complexes were analyzed by real-time blood circulation monitoring-EPR, enabling us to better understand the disposition of the paramagnetic species in the blood (Fig. 5A).  

VOSO₄ and the complexes were administered by a single intravenous (i.v.) injection to the rat under anesthesia. EPR spectra of the circulating blood were then measured at room temperature every 30 seconds. Disappearances of the vanadyl EPR signal in the blood were plotted against time after administration of the complex. The data was then analyzed by compartment models (Fig. 5B). The results revealed that the clearance rate of the vanadyl species in rats given VOSO₄ is higher with regard to half-life (t₁/₂) than that in those given several types of vanadyl complexes. The rate was 5 minutes in VOSO₄-treated rats and 7–30 minutes in the vanadyl complex-treated rats.  

We then investigated the absorption processes of VOSO₄, VO(pic)₂, and VO(6mpa)₂ in the gastrointestinal tracts of rats when administered by i.p. injection. We also monitored these processes in the stomach, jejunum, and ileum by EPR. The bioavailability (Fa, absorption rate) of the compounds was enhanced in the following order after oral administration, VO(6mpa)₂ → VO(pic)₂ → VOSO₄. The bioavailability of VO(6mpa)₂ on ileum administration was enhanced more than at other administration sites, resulting in a 1.8-fold increase compared with oral administration (Table 3).  

### Treatment of DM Subjects by VOSO₄ and a Proposal for the Use of Enteric-Coated VOSO₄ Capsules

When administered orally at a dose of 150 mg day⁻¹ for 6 weeks, VOSO₄ was shown to be advantageous in treating the subjects with type 2 DM in terms of plasma glucose, hemoglobin A₁c (HbA₁c: glucocyl hemoglobin, an index of the blood glucose control in diabetic patients for approximately one month), and fructosamine (an aminosugar, an index of the blood glucose control for approximately 2 weeks) levels. Before treatment, plasma vanadium levels were below 10 µg L⁻¹ but increased to 104 ± 18 µg L⁻¹ after 6 weeks of VOSO₄ administration as analyzed by atomic absorption spectroscopy. These results strongly indicate the importance of plasma vanadium levels with regard to antidiabetic activity during VOSO₄ treatment.

The bioavailability (Fa) of VOSO₄ following a bolus oral administration in rats was 4.8% for the active form of vanadyl species in rat blood estimated by EPR. However, Fa of the vanadyl species was enhanced approximately two and three
times when VOSO₄ was administered in the jejunum and ileum, respectively. These results prompted us to examine whether VOSO₄ should be administered directly to such sites in rats. For this purpose, we prepared enteric-coated capsules (ECCs) containing solid VOSO₄, administered them to rats, and then monitored the vanadyl levels in the blood. Interestingly, the Fa (9.8%) of VOSO₄ by ECC administration was almost double that of VOSO₄ administered from either gelatin capsules (4.0%) or solution (4.8%) (Table 4).

Thus, the administration of ECCs containing VOSO₄ to diabetic patients was found to improve vanadyl absorption, which in turn will normalize plasma glucose levels faster than through the administration of VOSO₄ solution or gelatin capsules.

Table 3. Metallokinetic parameters in the absorption processes of VOSO₄, VO(pic)₂, and VO(6mpa)₂, after oral, intrajejunal and intraileal administrations [a].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Administration site</th>
<th>AUC (nmol·hr/mL)</th>
<th>Cmax (nmol/mL)</th>
<th>MRT (hr)</th>
<th>Tmax (hr)</th>
<th>Fa [b] (%)</th>
<th>Enhancement of Fa</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOSO₄</td>
<td>stomach</td>
<td>165 ± 6</td>
<td>18.9 ± 5.2</td>
<td>7.93 ± 0.12</td>
<td>8.33 ± 3.79</td>
<td>4.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>jejunum</td>
<td>348 ± 5</td>
<td>47.8 ± 7.4</td>
<td>5.20 ± 0.03</td>
<td>2.67 ± 1.53</td>
<td>10.1</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td>ileum</td>
<td>433 ± 57</td>
<td>31.5 ± 12.9</td>
<td>9.09 ± 0.41</td>
<td>1.50 ± 0.87</td>
<td>12.6</td>
<td>2.62</td>
</tr>
<tr>
<td>VO(pic)₂</td>
<td>stomach</td>
<td>223 ± 12</td>
<td>26.2 ± 3.4</td>
<td>6.87 ± 0.09</td>
<td>7.00 ± 1.00</td>
<td>5.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>jejunum</td>
<td>442 ± 23</td>
<td>45.2 ± 1.2</td>
<td>8.13 ± 0.28</td>
<td>4.67 ± 0.58</td>
<td>10.5</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>ileum</td>
<td>454 ± 43</td>
<td>56.6 ± 8.3</td>
<td>7.44 ± 0.57</td>
<td>4.00 ± 1.73</td>
<td>10.8</td>
<td>2.04</td>
</tr>
<tr>
<td>VO(6mpa)₂</td>
<td>stomach</td>
<td>388 ± 52</td>
<td>54.6 ± 9.7</td>
<td>7.60 ± 0.20</td>
<td>5.50 ± 0.58</td>
<td>9.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>jejunum</td>
<td>311 ± 65</td>
<td>53.0 ± 3.6</td>
<td>7.30 ± 1.00</td>
<td>6.50 ± 0.58</td>
<td>7.8</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>ileum</td>
<td>699 ± 9</td>
<td>99.3 ± 4.9</td>
<td>6.82 ± 0.11</td>
<td>5.00 ± 0.00</td>
<td>17.6</td>
<td>1.80</td>
</tr>
</tbody>
</table>

[a] Data are shown as the mean values ± S.D. for three or four rats.
[b] Enhancement of Fa is calculated as [Fa value of rats given each compound after each administration sites/that after oral administration].
Abbreviations: AUC: area under the vanadyl concentration in the blood-time curve; Cmax: maximal vanadyl concentration; MRT: mean residence time of vanadyl species; Tmax: time required to attain Cmax; Fa: absorption ratio.
Mechanism of Insulin-Mimetic Action of Vanadium

Because vanadate(VO$_3^-$) behaves in a way similar to phosphate (PO$_4^{3-}$), the *in vitro* effects of vanadium are understood to inhibit protein phosphotyrosine phosphatase, which follows stimulation of protein tyrosine phosphorylation. Vanadate has also been reported to activate autophosphorylation of solubilized insulin receptors in a way that is similar to the action of insulin. 46–48 Vanadate also stimulates the tyrosine kinase activity of the insulin receptor β subunit. 49,50 In addition, both vanadate and vanadyl have been found to be effective in stimulating glucose metabolism in rat adipocytes. 46,47

Other evidence of the efficacy of vanadate comes from the observation that vanadate restores the expression of the insulin-sensitive glucose transporter of the skeletal muscles in rats and induces the recruitment of the GLUT4 glucose transporter to the plasma membrane of the adipocytes. 51,52

In addition, the effects of vanadium on lipid metabolism were examined. The adenosine 3',5'-cyclic monophosphate (c-AMP)-mediated protein phosphorylation cascade in adipocytes has been found to be activated during diabetes (*in vivo*) or in the presence of adrenaline (*in vitro*), and both glucose and vanadyl, which are incorporated in the adipocytes in response to vanadyl treatment, lead to the restored regulation of this cascade in peripheral (not localized or whole body) cells. 53–55 Thus, FFA release from the adipocytes is thought to be inhibited by vanadyl. The suppressed FFA release by vanadate depends on the enhancement of glucose incorporation by the metal ion, which is reduced to vanadyl by the added glucose. Therefore, vanadate is thought to not inhibit the release of FFA in the adipocytes in a dose-dependent manner, with this effect in the presence of glucose being completely reversed by an inhibitor of the glucose transporter, Cyt B. However, these effects are not seen in the absence of glucose. It was therefore suggested that glucose, which is incorporated in the adipocytes by the action of either insulin or vanadyl, suppresses the release of FFA. When other inhibitors, such as HNMPA-(AM)$_3$, hydroxy-2-naphthalenylmethylphosphonic acid tris-acetoxymethylester), wortmannin, and cilostamide for insulin receptor tyrosine kinase, phasphatidylinositol 3-kinase, and phosphodiesterase, respectively, were tested, only cilostamide restored the inhibited release of FFA by vanadyl. Based on these observations as well as the results of the interaction of the adipocytes with the VO(opt)$_2$ complexes,22 we have proposed a possible mechanism by which vanadyl acts on at least three sites, such as phosphatidylinositol 3-kinase (PI-3 kinase), glucose transporter, and phosphodiesterase in cells to normalize both the glucose and FFA levels in diabetic rats, as shown in Figure 1.

Recently, interesting *in vivo* effects of the VO(ma)$_2$ complex have been reported. Although insulin was found to increase protein kinase β (PKβ) activity in both STZ rats and fatty Zucker rats, the VO(ma)$_2$ complex produces no effects on PKβ activity. 56 In addition, the VO(ma)$_2$ complex has no apparent effects on PI-3 kinase activity in both STZ rats and fatty Zucker rats. 57 These results indicate the need for examining the action mechanisms of metal complexes in *in vivo* experiments.

### Possibilities for Preventing the Onset of DM by Vanadium

A novel hypothesis has been proposed in which nitric oxide (NO) production from macrophages (mø: giant cells with phagocytosis) mediates autoimmune destruction of islet B-cells of type 2 DM. An immunosuppressant, cyclosporin A, and a poly(ADP-ribose)polymerase inhibitor, nicotinamide, extend the remission phase and preserve the functioning of islet B-cells in patients. The relationship between mø and NO relative to DM has been investigated extensively. 58 In experimental animals, cyclosporin A has been reported to inhibit NO synthesis in murine mø and to prevent the toxic action of NO on islet B-cells *in vitro*. Nicotinamide inhibits inducible NO synthase (iNOS) in murine mø. In addition, administration of NO synthase inhibitors N$^\text{°}$-monomethyl-L-arginine (L-NMMA) and N-nitro-L-arginine-methylester (L-NAME) prevent the induction of DM by the administration of low doses of STZ. Isolated peritoneal mø increases NO production in STZ mice. In addition, peritoneal mø also increases NO
production in experimental animals such as BB rats and NOD mice that have spontaneously developed DM.

These observations suggest that NO released from mφ plays an important role in B-cell destruction in type 1 DM. It appears that suppressing the release of NO from mφ during the prediabetic phase prevents the onset of DM.

To clarify the mechanism of the antidiabetic activity of VOSO₄, NO production from peritoneal mφ of diabetic mice in which DM had been induced with low doses of STZ was monitored after the mice had received a VOSO₄ injection. Changes in the serum glucose levels of BALB/c mice with STZ-induced DM (STZ mice) were monitored following daily i.p. injections of VOSO₄. To avoid the possibility of a direct chemical reaction of STZ and vanadyl in mice, the administration of VOSO₄ was started 48 hours after the final STZ treatment. BALB/c mice that had received daily STZ injections at a dose of 40 mg/kg for the first 5 days developed diabetes (glucose approximately 200 mg/dL serum) on day 6 following the discontinuation of STZ administration. However, STZ mice that had received VOSO₄ at a dose of 10 mg/kg of body weight for the first 2 days followed by 5 mg/kg for the next 5 days maintained serum glucose levels in the normal range (glucose approximately 150 mg/dL). Mice that had received only VOSO₄ had no significant changes in serum glucose levels. Furthermore, the administration of VOSO₄ for 6 days to STZ mice partially accelerated the decrease in serum insulin. These observations indicate that daily administration of VOSO₄ is effective in preventing the onset of STZ-induced diabetes in mice.

We therefore speculated that the effects of VOSO₄ opposing the onset of diabetes relate to the function of mφ and to the possibility that mediator molecules such as NO play an important role in pancreatic B-cell destruction. To examine the effects of VOSO₄ on the peritoneal mφ of STZ mice, the NO production of peritoneal mφ was determined in the presence of VOSO₄. The inhibition of NO production from isolated peritoneal mφ activated with interferon-γ (IFNγ) plus lipopolysaccharide (LPS) depended on the level of the VOSO₄ dose.

As such, the administration of VOSO₄ during the prediabetic phase was expected to inhibit NO production from peritoneal mφ. The in vivo effect of VOSO₄ on NO production in the mφ of STZ mice was examined. NO production was enhanced in peritoneal mφ of STZ mice compared with that of normal mice, but NO production was significantly suppressed in the peritoneal mφ of STZ mice that had received VOSO₄. However, normal mice that had received only VOSO₄ had enhanced NO production. Given during the prediabetic phase of STZ mice, VOSO₄ suppressed NO production in peritoneal mφ to normal levels indicating that VOSO₄ and peritoneal mφ functions are closely related to the vanadium-dependent inhibition of the onset of DM.

Based on these results, a possible mechanism for vanadium-dependent prevention of the onset of diabetes onset was proposed (Fig. 6): mφ (indicated as ND-mφ) of normal mice treated with VOSO₄ is relatively low in the incorporation of vanadium and in enhanced NO production. In contrast, in the prediabetic phase of mice treated with low doses of STZ, activated mφ exudes through the islets, and a cytotoxic mediator such as the NO produced by the activated mφ destroys normal islet B-cells. In addition, a mechanism for the onset of DM by STZ administration was proposed based on the enhancement of the generation of superoxide anions (O₂⁻) in islet B-cells. Thus, it was assumed that NO reacts with the generated O₂⁻ to form peroxynitrite, ONOO⁻. Because one of the degradation products of peroxynitrite is a hydroxyl radical (·OH), free radicals such as ·O₂⁻ and ·OH are thought to destroy normal islet B-cells. However, mφ (indicated as D-mφ) of low-dosed STZ mice treated with VOSO₄ suppressed NO production. Suppression of cytotoxic mediators such as NO and ·OH preserved the damage to the islet B-cells in the prediabetic phase. Thus, vanadium-dependent modulation of immune responses appears to be responsible for the suppression of NO production.

Based on the above results, it appears that VOSO₄ suppresses excess NO production from mφ induced during the prediabetic phase by STZ treatment in low doses. This finding may be useful with regard to the clinical applications of VOSO₄ and vanadyl complexes in preventing the onset of DM.58,59

In conclusion, based on our results with experimental diabetic animals, several vanadyl complexes have been proposed
as effective not only for treating both types of DM but also for preventing the onset of DM. We know that VOSO₄ is effective in improving the health of subjects with DM. It may therefore be possible to use the vanadyl complexes proposed here in the treatment of DM patients because of their high degree of bioavailability and their effectiveness at lower doses than that of the doses of VOSO₄ used clinically.

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