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A new candidate for insulinomimetic vanadium complex: synergism of oxovanadium(IV)porphyrin and sodium ascorbate

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Abstract—Vanadyl-*meso*-tetrakis(1-methylpyridinium-4-yl)porphyrin, VOTMpyP with the $VO(N_4)$ coordination mode, was found to have a potent insulinomimetic activity on the basis of in vitro and in vivo experiments. When the complex was given simultaneously with sodium ascorbate, the high blood glucose levels of type 1 diabetic model STZ-rats were lowered by synergistic effect, probably sustaining the vanadyl state by means of ascorbate distributed in the organs and tissues of animals. This is the first finding on not only the insulinomimetic vanadyl–porphyrin complex but also the occurrence of a synergistic effect of VOTMpyP and sodium ascorbate to lower the high blood glucose levels in diabetic animals.

An in vitro finding in 1980 of augmentation by vanadate (+5 oxidation state of vanadium) of glucose incorporation in the isolated rat adipocytes opened a research window on a new physiological role of vanadium.[1] Two research groups found insulinomimetic activity of vanadate in reports appearing in 1985 and 1987, in which the metal ion dissolved in drinking water lowered the high blood glucose levels in streptozotocin (STZ)-induced insulin-dependent type 1 diabetic rats (STZ-rats).[2,3] In consideration of the fact that vanadate is more toxic than vanadyl ion (VO^{2+}, +4 oxidation state of vanadium) to rats,[4] and the fact that administered vanadate is reduced to vanadyl in rats,[5] we attempted to develop new insulinomimetic vanadyl complexes that are more active and less toxic than either vanadate or vanadyl. In 1990, we found that vanadyl-cysteinemethylester complex given by daily oral administrations normalizes the blood glucose levels in streptozotocin (STZ)-induced type 1 diabetic rats (STZ-rats).[6] Since then, many types of insulinomimetic vanadyl complexes with different coordination modes around vanadyl, such as $VO(O_4)$, $VO(S_4)$, $VO(S_2O_2)$, $VO(S_2N_2)$, $VO(N_2O_2)$, and $VO(N_3O)$, have been proposed,[7] however, few complexes with the $VO(N_4)$ coordination mode have been examined. In 1999, Woo et al. reported an insulin-enhancing vanadyl–biguanide (biguanide, metformin, and phenformin) complexes with the $VO(N_4)$ coordination mode.[8] Among those, bis(metformin)-oxovanadium(IV) complex was found to significantly lower the blood glucose levels of STZ-rats. To develop other types of complexes with the $VO(N_4)$ coordination mode having this pharmacological activity, we used vanadyl-*meso*-tetrakis(1-methylpyridinium-4-yl)porphyrin (VOTMpyP) as a stable complex.[9]

Recently, it was revealed that ascorbic acid metabolism is impaired in STZ-rats; ascorbate concentrations in the plasma, liver, and kidney of STZ-rats were significantly lower than those of the control, and these concentrations decreased further as the diabetic state continued.[10] Those results suggest that the pathophysiological states of STZ-rats change with respect to the ascorbate concentration, and that, consequently, when a vanadyl complex is given to STZ-rats, the complex might be oxidized in part to the vanadate state. Thus, it is expected that simultaneous administration of a vanadyl complex and ascorbic acid is an effective method by which the active form of the insulinomimetic vanadyl state of the complex is sustained in the organs and tissues. When

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VOTMpyP and sodium ascorbate were simultaneously given to STZ-rats, the high blood glucose levels were lowered, suggesting the occurrence of a synergistic effect of these two compounds. This paper is the first, to our knowledge, to report not only the in vitro and in vivo evaluations of the insulinomimetic effect of VOTMpyP but also the occurrence of a synergistic effect of VOTMpyP and sodium ascorbate to lower the high blood glucose levels.[11]

In vitro insulinomimetic activity was examined with respect to 50% inhibitory concentration (IC_{50}) of FFA (free fatty acids) release from isolated rat adipocytes.[12] VOTMpyP ($IC_{50} = 3.38 \pm 0.81$ mM) was less active than $VOSO_4$ ($IC_{50} = 1.00 \pm 0.34$ mM). Significantly lowered IC_{50} of $VOSO_4$ with sodium ascorbate (0.34 ± 0.15 mM) strongly indicated that the vanadyl species added as $VOSO_4$ is oxidized during the incubation and the vanadyl state sustained by ascorbate is a true active form to exhibit insulinomimetic activity, whereas IC_{50} of VOTMpyP with sodium ascorbate was almost the same as that without ascorbate. In addition, it was found that the vanadyl species of VOTMpyP in 4% BSA (bovine serum albumin, fraction V, Sigma) as monitored by EPR at 77 K was stable for 6 h irrespective of the presence or absence of sodium ascorbate, whereas that of $VOSO_4$ was oxidized over that time period but stabilized in part with ascorbate (Fig. 1).

Next we investigated whether VOTMpyP is active in diabetic STZ-rats by bolus intraperitoneal (ip) injection at a dose of 5.0 mg V/kg of body weight, and found that the concentration of the complex lowers transiently the blood glucose level for 2–3 h after administration (Fig. 2), suggesting that the complex may be excreted quickly.

In fact, the urinary clearance curve of vanadyl species in STZ-rats that received VOTMpyP at a dose of 3.5 mg V/kg of body weight indicated that approximately 50% of the administered complex is excreted by 5 h after ip injection (Fig. 3). Interestingly, the intensity of the EPR spectrum due to vanadyl species in the urine of STZ-rats given VOTMpyP for 1 hr was augmented 1.6 fold after addition of sodium ascorbate, without alteration in the spectral shape (Fig. 4), indicating that approximately 40% of VOTMpyP was oxidized to the vanadate form or lost the paramagnetism, in which the chemical structure is as yet unknown. These results led us to use combined administration of VOTMpyP and sodium ascorbate in STZ-rats.

When VOTMpyP and sodium ascorbate were given individually at a daily dose of 2.0 mg V/kg and 20 mg/kg, respectively, no changes in the blood glucose levels were observed. In contrast, when these two compounds were administered simultaneously from 10th day after treating with VOTMpyP, the high blood glucose levels were lowered (Fig. 5), reflecting a synergistic effect.

To understand the synergistic effect of the compounds, metallokinetic analysis and vanadium distribution in organs[13] in rats were examined in terms of vanadium concentration by using blood circulation monitoring-EPR[14] and neutron activation analysis (NAA), respectively.

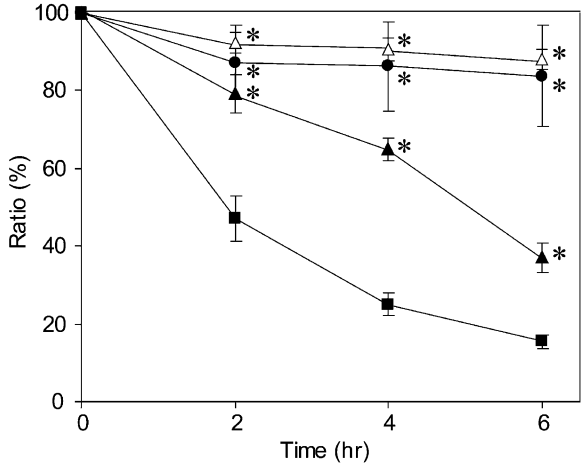

Figure 1. Time-dependent changes of EPR signal intensities due to vanadyl species of 1 mM $VOSO_4$ (■), $VOSO_4$ + 1 mM sodium ascorbate (▲), 1 mM VOTMpyP (●), and VOTMpyP + 1 mM sodium ascorbate (△) in 4% BSA solution ($n = 3$) at each time for those at 0 h at 37 °C. Significance; * $p < 0.01$ versus $VOSO_4$ at the same time.

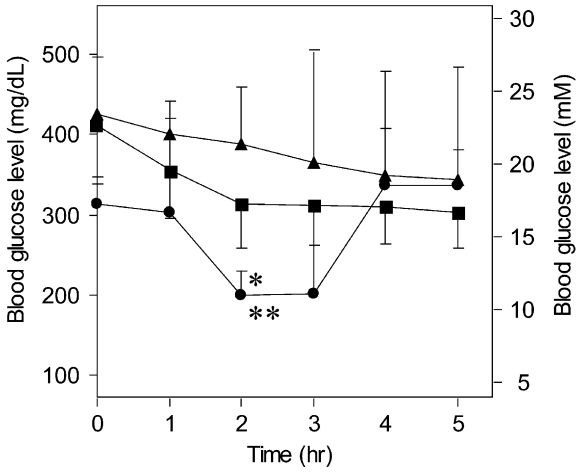

Figure 2. Changes of blood glucose levels in STZ-rats following acute ip injection of saline (▲, $n = 5$), $VOSO_4$ (■, $n = 5$), and VOTMpyP (●, $n = 3$) at a dose of 5.0 mg V/kg. Significance; *$p < 0.05$ versus $VOSO_4$ at the same time, **$p < 0.01$ versus saline at the same time.

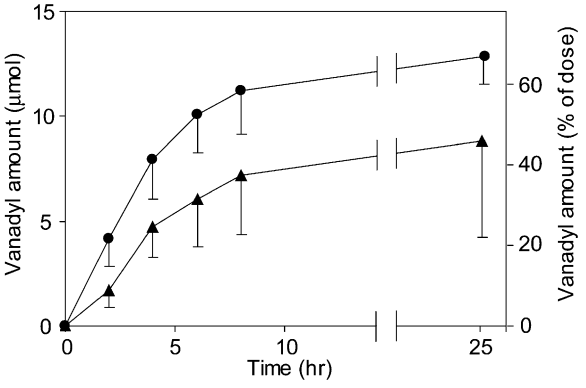

Figure 3. Cumulative amounts of vanadyl species in the urine of rats, who received ip $VOSO_4$ (▲, $n = 4$) and VOTMpyP (●, $n = 4$) at a dose of 3.5 mg V/kg. Urine was collected at every 2 h from rats. Vanadyl concentrations in the urine were determined by X-band EPR.

Vanadyl species remained longer in the blood of rats receiving VOTMpyP by intravenous (iv) injection than in that of rats receiving iv injection of $VOSO_4$ (Fig. 6),[15,16] suggesting its stronger and longer hypoglycemic activity. Total vanadium was incorporated in almost all organs, where the levels of total vanadium were significantly higher in the liver and kidney than other organs (Fig. 7). Thus, vanadium distributed in organs was indicated to contribute to the development of the insulinomimetic activity in STZ-rats.

Although STZ-rats have been reported to be in the state of ascorbic acid deficiency,[10] the administration of sodium ascorbate did not show the therapeutic effect for the diabetic state (Fig. 5). Thus, the administration of ascorbate was assumed to improve the redox state of STZ-rats. On the other hand, VOTMpyP, which was oxidized in part during the metabolism in STZ-rats, had no insulinomimetic activity. However, simultaneous administration of VOTMpyP and sodium ascorbate was effective to lower the high blood glucose levels of STZ-rats. This observation was thought to be a kind of synergistic effect, where the vanadyl state of VOTMpyP was sustained or the metabolic decomposition of VOTMpyP was inhibited by co-administration of sodium ascorbate. In the connection of our present observation, an interesting result was reported in which zinc(II)–ascorbate complex improved the diabetic state of KK-A^y mice with type 2 diabetic mellitus.[17]

In conclusion, VOTMpyP with the $VO(N_4)$ coordination mode was found to have a potent insulinomimetic activity, and when the complex is given simultaneously with sodium ascorbate, the high blood glucose levels of diabetic STZ-rats are lowered by synergistic effect, probably sustaining the vanadyl state by means of ascorbate distributed in the organs and tissues of animals. This is the first finding on not only the insulinomimetic vanadyl–porphyrin complex but also the occurrence of a synergistic effect of VOTMpyP and sodium ascorbate to lower the high blood glucose levels in diabetic animals. The results will be important for analyzing the relationship between metabolism and insulinomimetic activity of VOTMpyP in diabetic animals.

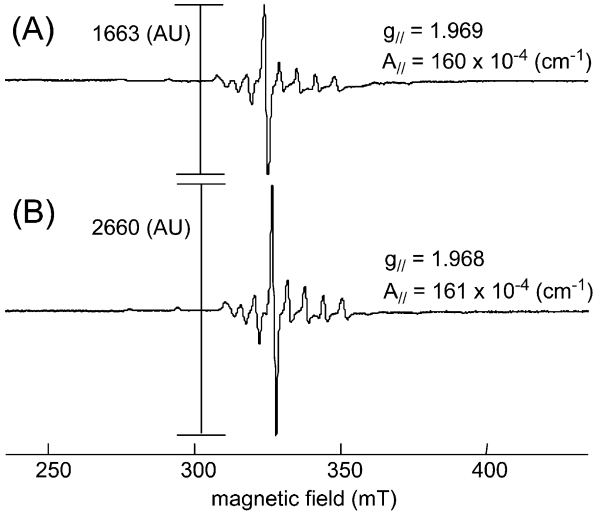

Figure 4. Change of EPR signal intensities (77 K) of the urine (A) and urine plus sodium ascorbate (B). The urines of STZ-rats were collected at 1 h after *ip* injection of VOTMpyP.

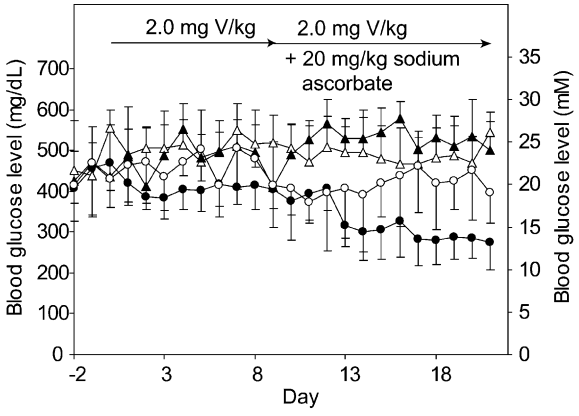

Figure 5. Changes of blood glucose levels in STZ-rats given saline (▲, $n=4$), sodium ascorbate (△, $n=6$), TMpyP (○, $n=4$), and VOTMpyP + sodium ascorbate after 10th day (●, $n=9$) by daily ip injections.

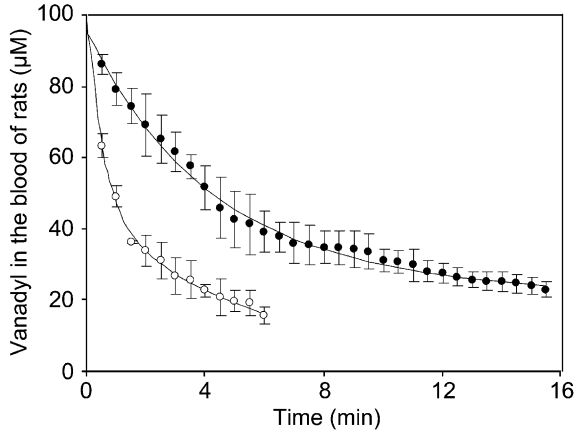

Figure 6. Vanadyl clearance as monitored by BCM-EPR from the blood of rats (○; $VOSO_4$, ●; VOTMpyP), who received iv $VOSO_4$ and VOTMpyP at a dose of 0.5 mg V/kg under anesthesia ($n=4$).

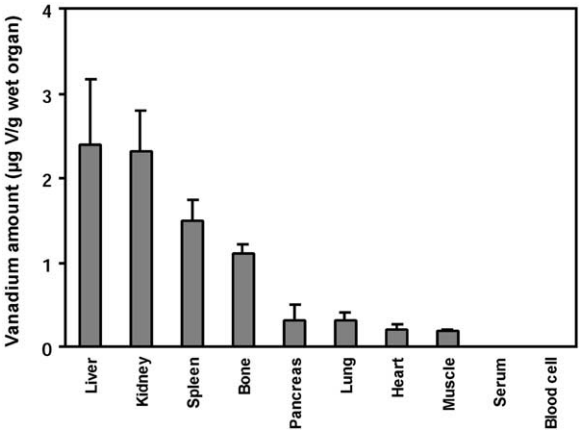

Figure 7. Organ distribution of vanadium in STZ-rats receiving daily ip injections of VOTMpyP at a dose of 2.0 mg V/kg for 3 weeks ($n=4$).

Acknowledgements

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References and notes

1. Dubyak, G. R.; Kleinzeller, A. *J. Biol. Chem.* **1980**, *255*, 5306.
2. Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. *Science* **1985**, *227*, 1474.
3. Meyerovitch, J.; Farfel, Z.; Sack, J.; Shechter, Y. *J. Biol. Chem.* **1987**, *262*, 6658.
4. Hudson, F. T. G. *Vanadium Toxicology and Biological Significance*; Elsevier: New York, 1996; p 140.
5. Sakurai, H.; Shimomura, S.; Fukuzawa, F.; Ishizu, K. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 293.
6. Sakurai, H.; Tsuchiya, K.; Nukatsuka, M.; Kawada, J.; Ishikawa, S.; Yoshida, H.; Komatsu, M. *J. Clin. Biochem. Nutr.* **1990**, *8*, 193.
7. (a) Sakurai, H.; Yasui, H.; Adachi, Y. *Expert Opin. Investig. Drugs* **2003**, *12*, 1189. (b) Sakurai, H.; Kojima, Y.; Yoshikawa, Y.; Kawabe, K.; Yasui, H. *Coord. Chem. Rev.* **2002**, *226*, 187. (c) Sakurai, H. *The Chem. Rec.* **2002**, *2*, 237. (d) Thompson, K.; McNeill, J. H.; Orvig, C. *Chem. Rev.* **1999**, *99*, 2561.
8. Woo, L. C.; Yuen, V. G.; Thompson, K. H.; McNeill, J. H.; Orvig, C. *J. Inorg. Biochem.* **1999**, *76*, 251.
9. Dougherty, G.; Pilbrow, J. R. *J. Chem. Soc., Faraday Trans. 2* **1985**, *81*, 1739.
10. Kashiba, M.; Oka, J.; Ichikawa, R.; Kasahara, E.; Inayama, T.; Kageyama, A.; Kageyama, H.; Osaka, T.; Umegaki, K.; Matsumoto, A.; Ishikawa, T.; Nishikimi, M.; Inoue, M.; Inoue, S. *Free Rad. Biol. Med.* **2002**, *33*, 1221.
11. VOTM*py*P was prepared and purified by a procedure in the literature,[9] by using TMpyP 6 tosylate (Dojindo) and $VOSO_4 \cdot 2.3H_2O$. The prepared complex was characterized by elemental analysis, IR (Shimadzu FTIR-8100A), visible absorption (Agilent 8453 UV–visible spectrometer), and electron paramagnetic (JEOL JES-TE1X X-band ESR spectrometer) spectra, and magnetic susceptibility (Sherwood Magnetic Balance HIC-1). Anal. calcd for $C_{44}H_{36}N_8OV(ClO_4)_4 \cdot 6H_2O$: C, 42.28; H, 3.88; N, 8.97%.
12. Found: C, 42.35; H, 3.85; N, 8.69%. $\nu_{V=O}$: 942 cm^{-1} (KBr disk). μ_{eff}(BM) = 1.75. λmax in nm (ε 1/M/cm): 439 (1.9×10^5), 563 (1.5×10^4), 603 (3.4×10^3) in H_2O. EPR parameters: $g_0 = 1.982$, $A_0 = 78\times10^{-4}$ cm^{-1}; $g_{//} = 1.966$, $A_{//} = 160\times10^{-4}$ cm^{-1}; $g_\perp = 1.990$, $A_\perp = 37\times10^{-4}$ cm^{-1}.
13. (a) Nakai, M.; Watanabe, H.; Fujiwara, C.; Kakegawa, H.; Satoh, T.; Takada, J.; Matsushita, R.; Sakurai, H. *Biol. Pharm. Bull.* **1995**, *18*, 719. (b) Isolated adipocytes (2.7×10^6 cells/mL) from Wistar rat epididymal fat pads were preincubated at 37 °C for 0.5 h with three different concentrations of vanadyl compounds in 1 mL KRB buffer (pH 7.4) with 20 mg BSA. A 10 μM epinephrine was then added and the solutions were incubated at 37 °C for 3 h. The reactions were stopped, and FFA levels in outer solution of the cells were determined with an FFA kit (Wako).
14. Total vanadium concentrations in the organs of rats given VOTMpyP at a daily dose of 2.0 mg V/kg for 20 days were determined for the lyophilized samples by using NAA at the Research Reactor Institute of Kyoto University.[12]
15. Yasui, H.; Tamura, A.; Takino, T.; Sakurai, H. *J. Inorg. Biochem.* **2002**, *91*, 327.
16. Metallokinetic parameters of VOTMpyP and $VOSO_4$ were obtained on the basis of two-compartment model as follows.[14] VOTMpyP: $AUC = 2.48 \pm 0.19$ (μmol·min/mL), $MRT = 70.3 \pm 17.3$ (min), $CL_{tot} = 4.0 \pm 0.3$ (mL/min/kg), $V_{ss} = 276 \pm 48$ (mL/kg), $t_{1/2}(\alpha) = 2.83 \pm 0.60$ (min), $t_{1/2}(\beta) = 54.2 \pm 11.9$ (min). $VOSO_4$: $AUC = 0.26 \pm 0.04$ (μmol·min/mL), $MRT = 4.4 \pm 0.7$ (min), $CL_{tot} = 38.9 \pm 5.8$ (mL/min/kg), $V_{ss} = 167 \pm 10$ (mL/kg), $t_{1/2}(\alpha) = 0.96 \pm 0.21$ (min), $t_{1/2}(\beta) = 3.0 \pm 0.5$ (min). All parameters of VOTMpyP were significantly different from those of $VOSO_4$ at the 1% level of Student's *t*-test.
17. Kojima, Y.; Yoshikawa, Y.; Ikura, N.; Ueda, R.; Ichimura, A.; Doe, M.; Sakurai, H. *Vitamins* **2003**, *77*, 571.