Role of Vanadium in Treating Diabetes

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Since insulin-dependent diabetes mellitus (IDDM), which causes many severe secondary complications, is characterized by hyperglyceria due to absolute deficiency of insulin, the diseases is controlled by daily injection of insulin. Therefore, the development of insulin replacements of mimetics upon oral administration is an important investigation. Recent studies indicate that vanadium, which is proposed to be one of essential trace elements in animals and humans, relates to both glucose and lipid metabolisms, and the metal in turn shows insulin-mimetic effect. Thus several types of vanadium complex have been proposed to be insulin mimetics. In 1990, we proposed first vanadyl-cysteinate complex, which normalized the blood glucose level of IDDM rats on oral administration. On the other hand, simple vanadium compounds such as vanadyl sulfate and sodium vanadate have been reported to be useful to treat human non-insulin-dependent diabetes mellitus (NIDDM). Based on the observations, we have developed several types of vanadyl complexes with different coordination modes such as VO(O), VO(N), VO(S), VO(O,N), VO(S,N) and VO(O,S), and found that vanadyl-methylpicolinate complex with long acting character and low toxicity is the most effective to treat IDDM as well as NIDDM rats, when administered orally. The mechanism was also studied with respect to the pharmacokinetic analysis and vanadium distribution in animals. J. Trace Elem. Exp. Med. 12:393–401, 1999.

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INTRODUCTION

Diabetes mellitus (DM) is mainly classified into two types, insulin-dependent DM (IDDM) and noninsulin-dependent DM (NIDDM), after the definition according to WHO [1]. The numbers of patients suffering from DM are increasing daily, probably due to changes in lifestyle and food. All types of DM involve absolute or relative insulin deficiency. To treat NIDDM, synthesized therapeutics involving sulfonylureas, sulfonamides, biguanides, and triglydazone have been developed and clinically used. However, as yet, IDDM can be controlled only by daily subcutaneous (sc) injections of insulin; otherwise, it causes such secondary complications as diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy. To avoid the pain and stress of sc insulin injections as well as the secondary complications, it is important...

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to develop an orally active insulin replacement or mimetic to maintain quality of life (QOL) of DM patients.

Recently, vanadium ions, including vanadyl (VO$^{2+}$, +4 oxidation state of vanadium) and vanadate (VO$_2^-$, +5 oxidation state of vanadium), and especially several types of vanadium complexes, have been reported to be effective via oral administration in experimental DM animals, such as streptozotocin(STZ)-induced diabetic rats (STZ-rats) [2–4]. More recently, we found that several vanadyl complexes on oral administration are effective to treat hereditarily diabetic-inducing animals such as KKAy mice [5]. In clinical trials, it has been proposed that hepatic and peripheral insulin sensitivities in patients with IDDM and NIDDM were improved by giving simple vanadium compounds, such as vanadyl sulfate (VOSO$_4$) and sodium vanadate (NaVO$_3$), about a century ago in France [6] and, now, in 1995–1996 [7–11]. These findings strongly indicate the need for investigations to establish safe and long-term effective vanadium compounds to treat DM. Here, we report our recent results on the study of developing orally active and long-term acting vanadium complexes.

**VANADIUM IN NATURE AND ITS PHYSIOLOGICAL ROLES**

Vanadium, which is a transition metal, atomic number 23, atomic weight 50.9415, was discovered by Sefström in 1831 and named by him for the goddess Vanadis of Scandinavian legend (Fig. 1). Vanadium is the 21st abundant element in the earth’s crust, the average concentration being at 35 ppm (µg/g), and is contained at 2 ppm (µg/g) in sea water. Since vanadium has a similar character to that of phosphate and is present in several oxidation states at +2 – +5, many types of compounds have been prepared. Compounds with a +5 oxidation state are most stable. Usually, vanadium

![Figure 1. Sefström and Vanadis. Reproduced with permission from: Weeks ME, Leicester, HM. Discovery of the elements, 7th edition. Journal of Chemical Education, American Chemical Society; 1968.](image-url)
ions bind with the oxygen atom to form oxo compounds such as VO$_3^-$ as vanadate and VO$^{2+}$ as vanadyl forms. The vanadic form of V$^{3+}$ is very unstable under air and oxidizes to vanadyl or the vanadate form [12–14].

Humans usually take vanadium at 10–60 $\mu$g through foods daily, and 50–200 $\mu$g of vanadium is estimated to be found in the human body. In each organ, vanadium is present at 0.01–1 $\mu$g and contributes to a wide variety of physiological roles. In tissues, ~90% of vanadium is bound with proteins and 10% is present as low molecular ionic forms [15].

The importance of vanadium was noted in 1971 pertaining to the growth of rats and chicks, but this has not been established in humans. As far as we know, the following five living systems contain vanadium: (1) blood cells in limited species of ascidian, where the occurrence of an aquo vanadic compound has been proposed, (2) amanitata as a species of mushroom contains a purple-blue vanadyl complex, amavadin, (3) limited species of brown algae contain a metalloenzyme, bromoperoxidase, in the vanadate form at the resting state, (4) some species of macro-organism contain a vanadium nitrogenase, and (5) limited species of polychaete wohm.

When vanadium compounds are administered to experimental animals, the metal is found in such organs as kidney, liver, pancreas, and born. Especially, the vanadate form exhibits toxicity ~10–15 times more than the vanadyl form in rats in terms of LD$_{50}$ value [16].

Extensive study has revealed biochemical and physiological activities of vanadium, and the characteristic roles are summarized in Figure 2 [15]. Among them, the most interesting and striking role is its insulin-mimetic or antidiabetic activity [2–4,17].

INSULIN-MIMETIC ACTION OF VANADIUM

In the late 1960s, interesting findings were reported: ouabain (G-strophanthin), which has been used as a cardiac glycoside, inhibits Na$^+$, K$^+$-ATPase and exhibits insulin-mimetic activity with respect to glucose transport and metabolism in rat adipocytes [15]. However, vanadate has been found to be a potent inhibitor of Na$^+$, K$^+$-ATPase; thereby, vanadate was detected in the ATP product from Sigma (St. Louis, MO) [18]. (It might be useful to note that not only ATP, but also bovine serum albumin (BSA) contain vanadate to microgram/g protein concentration [19,20].) In addition, vanadium has been found to be present exclusively in the vanadyl state in almost all organs of rats given NaVO$_3$ or VOSO$_4$ [21]. These facts and the selective toxicity of vanadium compounds in rats, led us to use a vanadyl compound, VOSO$_4$, to examine the insulin-mimetic activity in in vitro evaluation, since vanadyl is less toxic than vanadate and more stable than the vanadic form. As expected, VOSO$_4$ was found to be effective in showing insulin-mimetic activity in terms of inhibition of the free fatty acid (FFA) release from rat adipocytes (Fig. 3) [22], as well as incorporation of glucose [23] into the adipocytes. In this way, the normoglycemic effect of vanadyl compounds was examined.

NORMOGLYCEMIC EFFECTS OF VANADYL COMPOUND

When STZ-rats were administered VOSO$_4$ by daily single intraperitoneal (ip) injection, hyperglyceria was normalized within 2 or 3 days. As long as daily adminis-
tration was continued, normoglycemia was maintained. Although both serum glucose and FFA levels were improved and normalized, the insulin level did not improve, indicating that the action of VOSO₄ is not peripheral. In fact, similar levels of vanadium in normal STZ-rats and vanadyl-treated STZ-rats were found in almost organs examined, as determined by neutron activation analysis (NAA), which is the most reliable vanadium determination method in living systems. Results of glucose tolerance tests concluded that IDDM is indeed treated with the administration of VOSO₄ [22,23]. Orally administered VOSO₄ was also shown to be effective [24]. On the basis of these results, orally active and long-term acting vanadyl complexes have been developed.

**ORALLY ACTIVE AND LONG-TERM ACTING VANADYL COMPLEXES**

To develop orally active therapeutics of metal complexes, we had to take into account that they pass the stomach at a low pH value such as 1–2 and thus might decompose under such acidic conditions. Accordingly, we prepared several vanadyl complexes with different coordination modes to ascertain the stability, bioavailability, or gastrointestinal absorption of the complexes. The first notable result was that oral administration of vanadate, which was dissolved in drinking water, normalized blood glucose levels in STZ-rats [25]. However, our aim was to develop the therapeutics in vanadyl form. In 1990, we proposed the first orally active insulin-mimetic vanadyl complexes involving vanadyl-cysteine methylester, -malonate and -tartarate complexes, the normoglycemic actions of such complexes being dose-dependent [26].

Against the principle of Pearson’s hard and soft acids and basis (HSAB), which proposes the formation of stable complexes due to combinations such as Lewis hard
acid-hard base or Lewis soft acid-soft base [27], the vanadyl-cysteine methylester complex with a hard acid (VO$^{2+}$)-soft base (thiolate) combination and VO(S$_2$O$_2$) coordination mode forms stable coordination bonds between VO$^{2+}$ and thiolates as well as amino groups [28,29]. Encouraged with the results, we have proposed the vanadyl-dithiocarbamate complexes with VO(S$_4$) coordination mode as orally active complexes [30,31].

On the basis of these results, we began our investigation to ascertain the structure-activity relationship of antidiabetic vanadyl complexes with different coordination modes, as shown in Figure 4 [3,4].

Establishment of a clear structure-activity correlation is difficult due to lack of available data, since essential factors of the complexes, such as physico-chemical properties and electronic changes under physiological conditions, hydrophobicity, gastrointestinal absorption, organ and subcellular distributions of vanadium and its complexes, contribute to each other. Nevertheless, we found that vanadyl-picolinate and its related complexes are good reagents, after the in vitro evaluation using rat adipocytes in terms of FFA release and in vivo tests on STZ-rats by daily ip injection and oral administration of the complexes [32]. Among them, vanadyl-6-methyl-picolinate complex, which has a similar IC$_{50}$ value to that of the leading vanadyl-picolinate complex but a moderate partition coefficient, has good insulin-mimetic activity, not only by ip injections (3 mgV/kg body weight for the first 2 days, 2 mgV/kg for 2 days, 1 mgV/kg body for 10 days), but by oral administration (10 mgV/kg body weight for 10 days, 5 mgV/kg for 10 days) without body weight loss and toxicity in STZ-rats. It is interesting to note that: (1) the serum glucose normalizing effect of the complex was continued for at least 80 days after ceasing its administration, and (2) the insulin-mimetic effect was observed at lower doses than those for the leading vanadyl-picolinate complex. Thus the vanadyl-6-methyl-picolinate complex with a moderate partition coefficient and relatively good IC$_{50}$
value on FFA release from adipocytes is expected to be a potent and long-term acting insulin-mimetic compound to treat animals with IDDM [33].

On the basis of our results, we propose the vanadyl-6-methylpicolinate complex as a future therapeutic reagent. However, to develop a therapeutic compound, we must know its pharmacokinetic behavior.

**METALLOKINETIC ANALYSIS**

Recently, we proposed a new pharmacokinetic analysis method combined with an electron spin resonance (ESR) spectrometer and named in vivo blood circulation monitoring-ESR (BCM-ESR) (Fig. 5). Using rats under anesthesia, the behaviors of many stable spin probes with a five or six member ring were successfully analyzed [34]. With the in vivo BCM-ESR method, we examined the metallokinetic features of VOSO₄, vanadyl-picolinate and -6-methylpicolinate complexes [35].

These compounds were given by single intravenous (iv) injection to rats at 37°C under anesthesia with pentobarbital, and ESR spectra were measured at room temperature every 30 sec and the data entered into a computer. Disappearance of the ESR signal due to vanadyl species in the blood was plotted against time after administration of the compounds. It was analyzed by one or two compartment model methods, depending on the structure of the vanadyl compound. The real-time ESR analysis of vanadyl species revealed that the clearance rate of the vanadyl species from the blood
of rats given VOSO₄ was higher than those given vanadyl-picolinate or -6-methylpicolinate complex in terms of half-life ($t_{1/2}$), being 5 min in VOSO₄-treated rats and 10–15 min in vanadyl complex-treated rats. The slow clearance rate of the vanadyl species in rats given two vanadyl complexes suggests a high distribution of vanadium in rat organs, which in turn indicates the long-term acting normoglycemic effects even after their withdrawal from the STZ-rats.

The proposed metallokinetic analysis by the in vivo BCM-ESR method is useful, not only for a pharmacokinetic analysis of the vanadyl complex in experimental animals, which in the future may apply to human patients, but for understanding the results obtained from the diabetic patients reported recently [7–11].

**CONCLUSIONS**

During our investigations to learn the relationship between structure and insulin-mimetic activity for vanadyl complexes with various types of coordination modes, we found that the vanadyl-6-methylpicolinate complex is an orally active and long-term
acting potent compound. Its characteristics are supported by high distribution in rat organs as determined by the NAA method and by a slow clearance rate as analyzed by the in vivo BCM-ESR method, compared with VOSO₄. A biometal such as vanadium may prove useful for treating diseases such as diabetes.

REFERENCES