Current state for the development of metallopharmaceutics and anti-diabetic metal complexes

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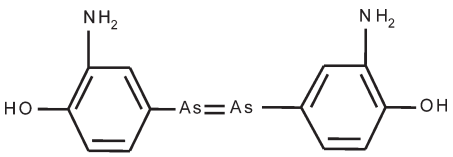
This *tutorial review* introduces the current state of metallopharmaceutics development by focusing on the topics of anti-diabetic vanadium and zinc complexes. Over thousands of years, people have produced many types of inorganic compounds, and the modern concept of chemotherapy was achieved by Ehrlich, who used an arsenic-containing compound to treat syphilis. Since then, many metallopharmaceutics have been developed worldwide. This review will be helpful to researchers who are interested in the current states of not only metallopharmaceutics but also anti-diabetic metal complexes.

1. Introduction: historical basis for developing inorganic pharmaceutics (metallopharmaceutics)

Since prehistoric times, inorganic compounds have been widely used to treat many diseases after a wide variety of experiences, attempts, and practices; for instance, mercury (Hg) was used for treating syphilis, magnesium (Mg) salts for intestinal treatments, and iron (Fe) salts for treating anemia. Most such compounds in those days were crude preparations obtained from minerals, plants, and animal sources; thus, people in those days could not imagine that inorganic compounds would be able to treat diseases.

The advances made in organic chemistry since the last half of the 18th century overshadowed interest in inorganic compounds bound with organic compounds (ligands). A very important concept was established in 1910; it was called "chemotherapy". Paul Ehrlich (1854–1915) and his co-workers discovered arsphenamine "Salvarsan", which is an organometallic compound with inorganic arsenic–carbon (As–C) bonds and spirochaeticidal activity. This compound and concept opened the way to develop a wide range of As compounds for the treatment of syphilis. Salvarsan was actually the first artificially prepared and clinically used pharmaceutic.[1]



Salvarsan

Ehrlich and his co-workers postulated that Salvarsan contained an As=As double bond; however, modern chemistry revealed the true structures of Salvarsan. Nicholson and colleagues reported that Salvarsan is a mixture of cyclic As–As bonded species. This mixture of trimers and pentamers serves to slowly release an $RAs(OH)_2$ (R = 3-amino-4-hydroxyphenyl) species, which probably gives rise to Salvarsan's anti-syphilitic activity.[1] The history of chemotherapy thus started with an inorganic element-containing compound. The As compounds owe their effectiveness to partial *in vivo* conversion to arsenoxides, which in turn react with the thiol groups (–SH) of some

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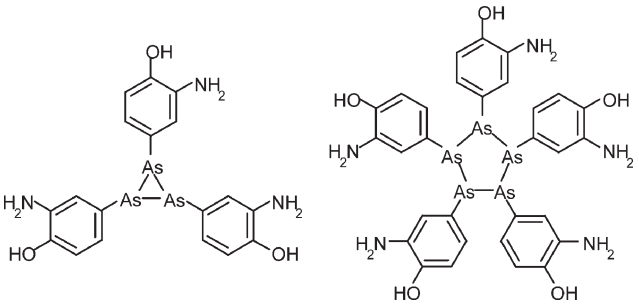
enzymes, particularly pyruvate oxidase and lypoic acid dehydrogenase, inducing inhibition of cellular function. The use of As compounds continued for approximately 30 years until the antibiotic "penicillin" was discovered by Alexander Fleming (1881–1955) and clinically used.



Through the time of Paracelsus (1493–1541), who proposed the application of several inorganic pharmaceutics containing Hg, Sb, Pb, Cu, As, S, B, and Ag to treat diseases, interest in inorganic pharmaceutics has slowly grown in European countries. Following the establishment of the "coordination theory" by Alfred Werner (1866–1919), a new concept of "metals and life" came into being with the finding of urease, which was the first crystallized enzyme, analyzed in 1926 and found to bind nickel (Ni) at the active center in 1975. Approximately thousands of metalloenzymes and metalloproteins are now crystallographically analyzed.

On the other hand, the concept of essential trace elements in humans and animals has been proposed, and health disorders due to deficiency of elements such as Fe, Zn, Cu, and Se in humans have been observed in several countries. In most cases, the supplementation of these elements alleviated disorders in human and animal health.

On the basis of this background, a wide variety of metal-containing agents, metallopharmaceutics, have been proposed, and some of them have been clinically used since the 20th century, as shown in Table 1.

Metal binding has been known to enhance the treatment effect of organic pharmaceutics; for instance, Zn enhances the anti-inflammatory effect of ibuprofen when compared to the ligand alone, and the anti-cancer effect of antibiotic bleomycin, which cleaves DNA in the nucleus of the cell, is explained by Fe^{2+} binding, which follows molecular dioxygen activation to generate reactive oxygen species involving superoxide anions and hydroxyl radicals. Although these examples of the metal-binding ability of ligands (drugs) in cells or organs have been widely reported, in this review, we focus on isolated metal complexes (coordination compounds) with therapeutic effect and potential.

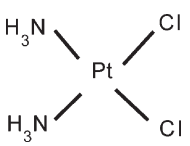
The most well-known example of the use of coordination compounds in the treatment of diseases is the application of platinum (Pt) complexes in cancer therapy. In 1969, Barnett Rosenberg and his co-workers observed the unusual phenomenon of filamentous growth of *E. coli* bacteria. This metamorphosis was induced by Pt^{2+} and Pt^{4+} ammine chloride complexes, which were generated *in situ* during electrolysis at the Pt electrodes, because the medium contained ammonium chloride. The compound responsible for the filamentation was *cis*-diamminedichloroplatinum^{2+} (cisplatin), which is a classic

Table 1 Established metallopharmaceutics and potential therapeutic coordination compounds

Element	Name	Year	Condition treated	Compound
Ag	Silver	1974	Burn and wound dressings	Silver-sulfadiazine, Slow-release Ag compounds
Al	Aluminium	1968	Stomach ulcer	Sucralfate
Au	Gold	1960	Rheumatoid arthritis	Auro-thiomalate, Auro-thioglucose, Auranofin
As	Arsine	1910	Syphilis	Arsphenamine
			Acute promyelocytic leukemia (APL)	Trisenol (Arseno trioxide)
Co	Cobalt	1940	Growth delay, anemia	Cyanocobalamin
Cu	Copper	2007	Antioxidant, UV-induced dermatitis	Copper-aspirinate[a]
Ge	Germanium	1985	Bacteria, cancers	Ge-132[a]
Li	Lithium	1954	Manic depressive psychoses	Lithium carbonate
Mo	Molybdenum	1981	Wilson's disease	Tetrathiomolybdate[a]
Pt	Platinum	1969	Cancers	Cisplatin
Se	Selenium	1998	Acute ischemic stroke	Ebselen
V	Vanadium	1990	Diabetes mellitus	Vanadium complexes[a,b]
Zn	Zinc	1990	Stomach ulcer	Polaprezinc
		2002	Diabetes mellitus	Zinc complexes[a,b]
		2003	UV-induced dermatitis	Zinc complexes[a]
		2007	Metabolic syndrome	Zinc-thioallixin-*N*-methyl and Zinc-dithiocarbamate complexes[a,b]

[a] Anticipated to be developed as pharmaceutics. [b] Will be described in the review.

coordination compound that was first prepared in 1844. Cisplatin has been found to be highly effective in treating tumors in not only animals but also humans with testicular, ovarian, lung, bladder, heart, neck, and cervical cancers.[2] Interestingly, its *trans* form, transplatin, is less effective in treating such cancers. The mechanism of the anti-cancer activity of cisplatin has been actively studied for many years; however, it is not yet fully understood. Nonetheless, new Pt complexes of the second and third generations involving polynuclear Pt complexes acting against cisplatin-resistant tumors have been proposed.



Cisplatin

On the other hand, reactive oxygen species (ROS) have been known to be involved in the pathogeneses of various diseases

such as lifestyle-related diseases, hypertension, and photo-aging due to exposure to ultraviolet (UV) light. Cu-dependent and Zn-modulated cytosolic and extracellular superoxide dismutases (Cu_2Zn_2SOD) exist in a wide range of mammals, and catalyze the dismutation reaction of the superoxide anion radical ($^{\bullet}O_2^-$) to yield hydrogen peroxide (H_2O_2) and triplet state oxygen ($^3O_2$). Because SOD activity in mammalian cells tends to decrease with an increase in age, the intake of SOD activity-enhancing compounds, which can facilitate *de novo* synthesis of Cu_2Zn_2SOD, is recommended. However, SOD is easily digested by gastric and intestinal proteases. Therefore, the importance of oral intake and skin ointments of small-molecular-mass SOD-mimetic metal (Cu, Mn, and Fe) complexes with active site structures similar to those in SOD enzymes has been proposed since 1974,[3] and research on the development of SOD-mimetics has been performed by many researchers.[4]

Among the many established examples involving Al, Au, Li, Pt, and Zn complexes (Table 1), we focus on the quite recent developments in potent anti-diabetic vanadium (V) and zinc (Zn) complexes[5] in this review.

2. Physiological activity and toxicity of vanadium and zinc

Physiology of vanadium

Vanadium (V), with atomic number 23, atomic weight 50.94, and oxidation states from +3 to +5, was discovered in 1831 by Sefström. In 1899, French physicians reported a clinical trial of V in diabetes. At that time, V was believed to be a panacea for human disorders and since then discussion has been continued for many years on whether or not V is nutritionally and pharmacologically important. Because V was proposed to be an essential nutrient in some animals by different research groups in 1971 and then in 1973, a wealth of results exists on the physiological roles of V in cells and organisms (Fig. 1).[5] However, the extent of its importance in humans and animals is still debated.[6]

Coincidentally, in 1977, V biochemistry was discovered when vanadate (+5 oxidation state of vanadium) was identified to inhibit sodium and potassium ATPase (Na^+-K^+-ATPase), because V was found in an ATP preparation derived from equine muscle.[7] Subsequently, the inhibition was proposed to have been caused by the substitution of phosphate with vanadate in ATP-driven reactions. This finding, which was confirmed by other researchers in 1978, increased the importance of V biochemistry and stimulated studies on V in many enzyme systems involving adenylate cyclase, tyrosine kinase, phosphotyrosyl phosphatase, and ribonuclease. Thus, V has been revealed to exhibit a wide variety of biochemical and physiological functions.

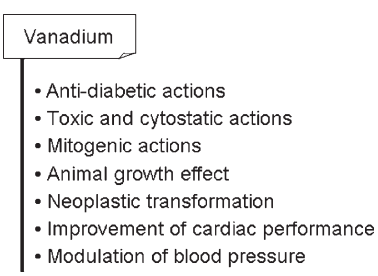


Fig. 1 Physiological roles of vanadium.[5]

V was originally examined as a possible pharmaceutic for treating syphilis, reducing serum cholesterol, and preventing caries, because the coenzyme A (CoA) content in the organs decreased with high doses of V.[6] One of the compounds involved in the synthesis of CoA is thioethanolamine, which is derived from the decarboxylation of cysteine. Hence, a decrease in cysteine caused by V was presumably the reason for the reduced CoA levels. CoA is involved in the synthesis of cholesterol and may therefore affect the occurrence of atherosclerosis.

Among many physiological roles of V, the insulinomimetic effect is the most striking; this effect is provided by the oxidation states +3, +4 (vanadyl, VO^{2+}), and +5 (vanadate, VO_3^- or VO_4^{3-}). One of the current focuses is to create pharmaceutics that will take advantage of the insulinomimetic and anti-diabetic properties of V in the place of insulin injections and synthetic drugs.[5,6] As described, French physicians reported in 1899 that sodium metavanadate ($NaVO_3$) partially improved the state of human patients with diabetes mellitus (DM), before the discovery of insulin in 1922 by Banting and Best. In recent years, both $VOSO_4$ and $NaVO_3$ have been clinically examined to determine whether they improve human DM, although the absorption and incorporation of these inorganic salts are generally very low. In addition, $VOSO_4$ is less toxic to rats than vanadate compounds,[8] and most V in the organs of normal rats treated with vanadate is exclusively present in the vanadyl form. From these observations, in general, it follows that low-molecular-weight ligands for vanadyl could be used with the expectation of obtaining higher bioavailability and lower toxicity than vanadyl or vanadate alone in animals.[5]

Toxicity of vanadium in animals and humans

The no-observed-effect level (NOEL) of reproductive toxicity of $NaVO_3$ in male mice is estimated to be 40 mg kg^{-1} day^{-1}.[8] Thus, $NaVO_3$ given to adult rats at 5, 10, or 20 mg kg^{-1} day^{-1} did not influence their reproductive performance but produced toxic effects in the offspring. V-induced morphological changes in the kidneys of rats treated with an intraperitoneal injection of Na_3VO_4 at 10 mg kg^{-1} day^{-1} for 8 consecutive days were more pronounced with age.

Although most foods contain low concentrations of V (<1 ng g^{-1}), food is the major source of exposure to V for the general population. The absorption of V salts from the gastrointestinal tract is poor in humans, and the excretion of V in the kidneys is relatively rapid with a half-life of 20–40 h in urine. Therefore, the toxicity of V compounds is low in general.[8] The estimated V intake of the US population ranges from 10–60 μg V day^{-1}. $VOSO_4$ is a common supplement used to enhance weight training in athletes at doses of up to 60 mg day^{-1}. In humans, the threshold level for V toxicity is concluded to be approximately 10–20 mg V day^{-1}.[8]

Most of the toxic effects of V compounds include local irritation of the eyes and upper respiratory tract, rather than systemic toxicity. The clearly documented effect of exposure to dust of vanadium pentoxide (V_2O_5) is upper respiratory tract irritation characterized by rhinitis, wheezing, nasal hemorrhage, conjunctivitis, cough, sore throat, and chest pain, because soluble V_2O_5 is absorbed in the lung.[8]

Physiology of zinc

Zinc (Zn), with atomic number 30, atomic weight 65.41, and oxidation state +2, was discovered by Marggrab in 1746. In 1977, Zn was determined to be an essential trace element in all living systems, including animals and humans, in whom this metal plays a structural role in hundreds of Zn enzymes and thousands of protein domains. Unlike iron (Fe), of which 80% of the total 3 g in humans occurs in heme-iron proteins, the total 2.3 g of Zn in humans is distributed among thousands of proteins. The broad distribution of Zn has made it difficult to establish its biochemical and physiological roles; hence, the large number of Zn-dependent biological processes and interactions should be understood in order to appreciate the significance and implications of dietary imbalances of Zn. However, the potential of Zn for growth and development as well as the transmission of genetic messages was not determined until 1993, because the chemical properties of Zn do not exhibit color or magnetism.

There are now approximately 200 three-dimensional structures for Zn proteins, representing all six classes of enzymes and covering a wide range of phyla and species. Three primary types of Zn proteins and enzymes are known: structural, catalytic, and cocatalytic. The most common amino acids involved in these three types are His, Gln, Asp, and Lys. Unique Zn proteins were first discovered in the 1980s. The first transcription factor found in 1983 was identified as a Zn enzyme, which led to the introduction of the term DNA-binding finger protein in 1985.[9] Transcription factors have been found to regulate gene expression, and their essential feature is binding to a regulatory protein in the recognition sequence of a gene. Many proteins have been found to possess a Zn-containing motif that serves to bind the DNA embedded in their structure. Within 15 years of the first discovery of the zinc-finger protein, hundreds of proteins have been identified. In 1995, a Zn transporter, which participates in a homeostatic system in the cell, was discovered. Metallothionein (MT), which was discovered in 1957, was found to link Zn distribution to the redox state of a cell in 1998.[10] In 2000, a Zn-containing regulatory protein was found to have a role in neurotransmission.[11]

One of the major advances in the past decade has been reported as the discovery of a homeostatic system of proteins that controls cellular Zn by coordinating Zn import and export, distribution, and sensing of Zn status. The involvement of many proteins in homeostatic control increases the potential for variations in Zn metabolism due to mutations in these proteins. For example, acrodermatitis enteropathica, a genetic disorder of Zn absorption in humans and a fatal disease if untreated with Zn, is caused by a mutation in the Zn transporter hZip4 (human zinc importer 4).[12] Therefore, pharmacological doses of Zn are given for the treatment of acrodermatitis enteropathica to ascertain whether the patient has obtained sufficient Zn.

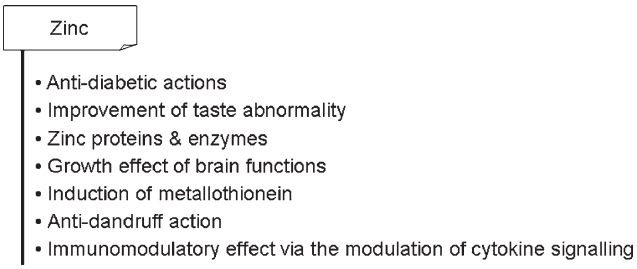


Fig. 2 Physiological roles of zinc.[5]

The physiological roles of Zn in the cells and organs are summarized in Fig. 2.[5] Zn is essential for growth and development. At the cellular level, it is critically involved in proliferation, differentiation, and apoptosis. Examples of Zn functions include immunity, intermediary metabolism, DNA metabolism and repair, reproduction, vision, taste, and cognition behavior. In addition, Zn is essential for neurogenesis, synaptogenesis, neuronal growth, and neurotransmission. It is stored in specific synaptic vesicles by a class of glutaminergic neurons and released as a neuromodulator in an activity-dependent manner.

Among the variety of physiological roles of Zn, its anti-diabetic activity is currently important. In fact, Zn and diabetes are linked at several points during metabolism in a cell.[13] With regard to the relevance of Zn to DM, Zn is contained in insulin and it binds with three nitrogen atoms from His and three H_2O molecules in an irregular octahedral environment, which is also believed to be a functional structure. Surprisingly, Zn was found to have important physiological and pharmacological functions involving insulinomimetic activity.[14] DM is also observed to accompany Zn deficiency. The accumulation of considerable clinical data is necessary because Zn has an insulinomimetic effect and inhibits the oxidative damage associated with DM. In this regard, Zn was found to stimulate rat adipocyte lipogenesis similar to the action of insulin; this was followed by observations of the *in vivo* anti-diabetic effects of oral $ZnCl_2$ in streptozotocin-induced type 1-like diabetic rats (STZ-rats) and ob/ob mice in 1992[15] and 1998,[16] respectively. However, in these observations, high doses or long-term administrations (8 weeks) of Zn were used, because the bioavailability of $ZnCl_2$ is relatively low. Then, the coordination compounds of Zn were prepared in order to enhance its bioavailability and therapeutic potential, and the first orally active anti-diabetic Zn complexes were discovered in 2002. Since then, a wide variety of insulinomimetic Zn complexes with different coordination environments around Zn have been proposed.[5,13]

Toxicity of zinc in animals and humans

Adverse effects have been reported to arise from either Zn deficiency or secondary deficiency of Cu due to excess

Zn. Food is a major factor inducing Zn deficiency, while Zn supplement is a major factor causing Zn toxicity. The recommendations for the guidelines of Zn intake are issued by various committees; however, these recommendations do not have precisely defined limits. Supplementation with quantities of Zn above the suggested upper limit can result in Cu deficiency, especially if the form of Zn in the supplement is readily absorbed from the gastrointestine.[17] However, the threshold for this effect is unknown. Literature provides many examples of Cu deficiency related to excessive supplemental Zn, thereby affecting many organs and functions. A supplement of 80 mg Zn day^{-1} is immunosuppressive and inhibits allogenic reactions. A supplement of 53 mg Zn day^{-1} impaired Cu status and behavior. Therefore, the amount of Zn supplement should be carefully considered. In particular, long-term supplementation with pharmacological amounts of highly absorbable forms of Zn, such as Zn-gluconate and Zn-acetate, should probably not be administered without close medical supervision.[17]

It appears that safe intake of highly absorbable Zn is related to the intake of Cu. Proportionately dietary and supplemental intake of Zn decreases the risk of Cu deficiency. Because the prevalence is now unknown, Zn intake should probably not exceed 20 mg Zn day^{-1} in adults.[17] In terms of severity, there are lethal effects, obvious clinical effects, and hidden effects. There seems to be no carcinogenic effect of high Zn intake. However, Zn deficiency as a risk factor for cancer and other diseases should be carefully observed in comparison with the adverse effects of high Zn intake.

3. Oxovanadium complexes with anti-diabetic activity

The insulinomimetic effect of V ions on cells and diabetic model animals has been reported since the 1980s.[18] In the last decade of the 20th century, V ions and their complexes were demonstrated to exert various insulinomimetic and anti-diabetic effects involving the enhancement of glucose transport and metabolism in isolated adipocytes and hepatocytes as well as skeletal muscle, stimulation of glycogen synthesis and lipogenesis, inhibition of lipolysis, and protein metabolism. Because inorganic V ions are generally considered to have poor bioavailability, the complexation of such V ions with organic ligands is of great interest with a view to reducing toxicity and improving bioavailability and tissue uptake of the ions. Among the three oxidation states of V ions, the oxovanadium(IV) form (vanadyl) has the advantage of exhibiting better insulinomimetic and anti-diabetic activities in terms of stability in cells, and toxicity and efficacy in animals. Thus, a large class of vanadyl complexes has been extensively proposed by many research groups.[5,13,19] The first generation of vanadyl complexes such as bis(methylcysteinato)oxovanadium(IV) ($VO(cysm)_2$) (1990),[20] bis(maltolato)oxovanadium(IV) ($VO(ma)_2$) (1992),[21] and bis(picolinato)oxovanadium(IV) ($VO(pa)_2$) (1995)[22] were found to normalize hyperglycemia (high blood glucose level) in streptozotocin-induced type 1-like diabetic rats (STZ rats). Following these findings, bis(acetylacetonato)oxovanadium ($VO(acac)_2$) was also shown to exhibit blood glucose lowering effect in 2000.[23]

To find more active and safer vanadyl complexes than the first generation of vanadyl complexes, a comprehensive study has been performed, where the *in vitro* and *in vivo* structure–activity relationships of bis(3-hydroxy-4-pyronato)oxovanadium(IV) ($VO(3hp)_2$) related complexes with a $VO(O_4)$ coordination environment were examined. From these studies, novel potent vanadyl complexes such as bis(ethylmaltolato)oxovanadium(IV) ($VO(ema)_2$) and bis(allixinato)oxovanadium(IV) ($VO(alx)_2$) were discovered (Fig. 3).[5]

In vivo anti-diabetic activities of the $VO(alx)_2$ were tested in terms of the hypoglycemic activity in diabetic model mice.[5] When the $VO(alx)_2$ complex was orally administered to KKA^y mice, which are obesity-linked type 2 DM model animals similar to human DM, for 4 weeks, an improvement was observed in both the blood glucose and HbA_{1c} levels (Fig. 4). In oral glucose tolerance tests (OGTT) after the treatment period, the blood glucose levels of the $VO(alx)_2$-treated KKA^y mice were almost equivalent to that in normal mice at 15 min after glucose loading. Plasma hyperinsulinemia in KKA^y mice was also completely normalized by the $VO(alx)_2$ treatment. These results indicate that $VO(alx)_2$ treatment improves insulin resistance and glucose intolerance in KKA^y mice. Based on these results, $VO(alx)_2$ was considered to normalize hyperglycemia in KKA^y mice by improving insulin resistance and glucose intolerance. On the other hand, a human trial using a vanadyl compound was reported, in which a 6 week oral $VOSO_4$ administration at a dose of 150 mg day^{-1} improved the hyperglycemic state in type 2 DM patients by reducing basal glucose production and enhancing muscle insulin sensitivity.[24] Following this observation, the first phase I trial of the $VO(ema)_2$ complex was reported in 2000.[25] The results for the nonlinear pharmacokinetics of $VO(ema)_2$ after oral intake have been reported, in which the C_{max} or AUC increased disproportionately with five oral doses (10–90 mg) and so the apparent oral clearance (dose/AUC) decreased significantly (5.4–1.5 L h^{-1}) as the dose was increased, suggesting

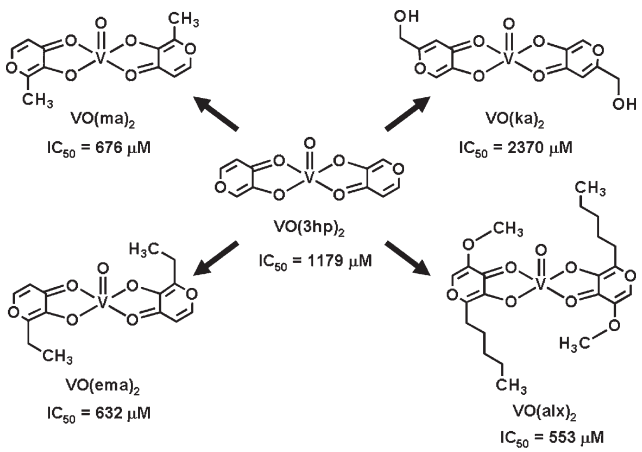


Fig. 3 Chemical structures of $VO(3hp)_2$ and its related complexes, and their estimated insulinomimetic activities (IC_{50} (metal complex concentration required to inhibit 50% of the free acids released from the isolated rat adipocytes treated with epinephrine): μM).

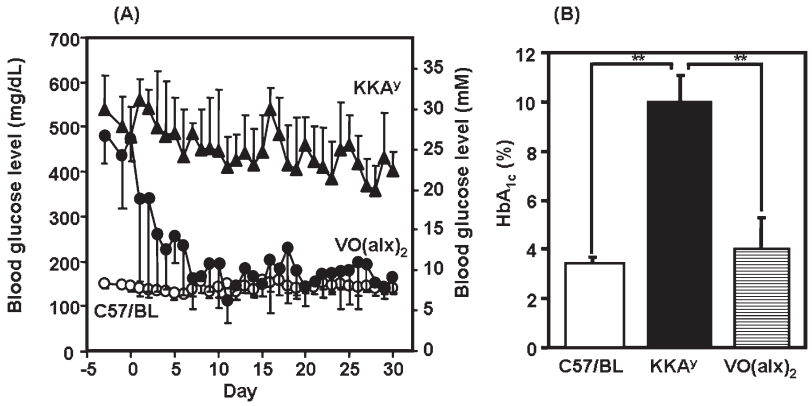
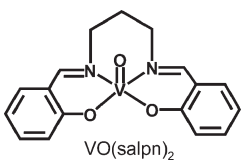
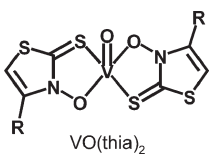
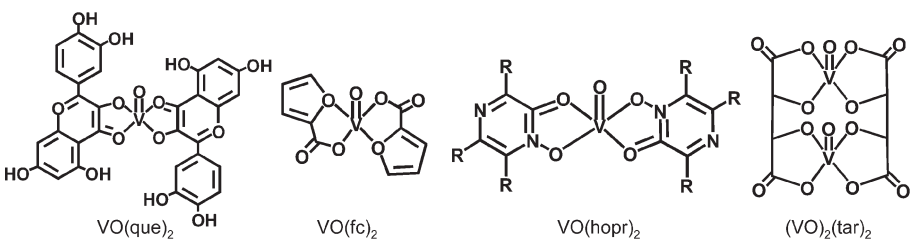
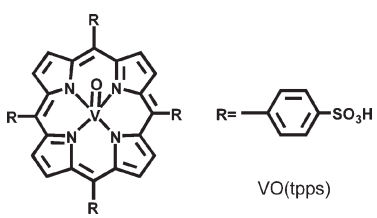


Fig. 4 Changes in blood glucose levels (A) in normal mice (C57/BL) and in KKA^y mice after daily oral administration of saline (KKA^y) or VO(alx)$_2$ for 30 days (doses were adjusted to maintain a concentration of 3–7 mg V kg^{-1} BW based on daily changes in the blood glucose level) and (B) amount of HbA$_{1c}$ after 30 days of administration. Data are expressed as the mean values ± SD for 4–6 mice. Significance: $*p < 0.05$ *vs.* C57/BL, $**p < 0.01$ *vs.* KKA^y mice.

that both the oral absorption and first-pass elimination of VO(ema)$_2$ are capacity-limited processes through the gastrointestine and liver, as previously described for several drugs in animals and humans. Other interesting anti-diabetic vanadyl complexes with different coordination environments, such as porphyrin complexes, thiazolethione complexes, hydroxydiazine-type complexes, furan complexes, quercetin-related complexes, salen-related complexes, and dinuclear complexes, have recently been proposed (Table 2).[5,26–28]

Table 2 Anti-diabetic new oxovanadium(IV) complexes with different coordination environments around vanadium[a]

N_2O_2	 VO(salpn)$_2$
S_2O_2	 VO(thia)$_2$
O_4	 VO(que)$_2$ VO(fc)$_2$ VO(hopr)$_2$ (VO)$_2$(tar)$_2$
N_4	 VO(tpps)

[a] Abbreviations: VO(salpn)$_2$: (*N*,*N*′-bis(salicylidene)-1,3-propanediaminato)oxovanadium(IV), VO(thia)$_2$: bis(3-hydroxy-thiazole-2(3*H*)-thionato) oxovanadium(IV), VO(que)$_2$: bis(quercetinato)oxovanadium(IV), VO(fc)$_2$: bis(α-furancarboxylato)oxovanadium(IV), VO(hopr)$_2$: bis(1-hydroxy-2(1*H*)-pyrimidinonato)oxovanadium(IV), (VO)$_2$(tar)$_2$: bis(L-tartrato)dioxovanadium(IV), VO(tpps): [*meso*-tetrakis(4-sulfonatophenyl)porphyrinato]-oxovanadium(IV).

4. Zinc complexes with anti-diabetic and anti-metabolic syndrome activities

Type 2 insulin-resistant DM accounts for 95% of all DM. Its worldwide frequency is predicted to grow by 6% per year, potentially reaching a total of 350 million cases in 2025. The main reason for the increasing incidence of DM is thought to be the increase in obesity—the most serious contributor to the pathogenesis of DM. It is now clear that the control of hyperglycemia in patients with type 2 DM can attenuate the development of chronic complications such as retinopathy and nephropathy. At present, the therapy for type 2 DM relies mainly on several chemotherapies intended to reduce hyperglycemia in addition to diet control and exercise. For instance, the therapy may include medicines such as sulfonylureas, which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production; thiazolidinediones, which enhance insulin action; and α-glucosidase inhibitors, which interfere with glucose absorption in the small intestine.[29] These therapies have limited efficacy, limited tolerability, and significant mechanism-based side effects. For example, a problem with sulfonylureas is that in many patients, who respond initially, the medicines become ineffective over time. Thus, newer approaches are urgently required. During the struggle to search for new pharmaceutics, several metal ions and their complexes were found to show anti-diabetic effects in experimental animals. In particular, studies on Zn were actively conducted. One of the remarkable features of Zn is its insulinomimetic activity and its potential link with insulin resistance and type 2 DM. Zn was found to stimulate lipogenesis and glucose transport in adipocytes; its supplementation in diet attenuated hyperglycemia in db/db mice. Clinical research showed evidence for a correlation between Zn deficiency and DM.[30] In DM studies, Zn is an important factor.

Zn actually exhibits insulinomimetic activity and an anti-diabetic effect; however, there has been little research regarding the anti-diabetic effect of Zn complexes. Quite recently, Zn complexes with excellent anti-diabetic and anti-metabolic syndrome effects in animals have been found; cyclo(His-Pro) and arachidonic acid plus Zn, Zn-allixin related complexes, and Zn-dithiocarbamate complexes act as anti-diabetic agents.[5,31]

Especially, bis(thioallixin-*N*-methyl)Zn ($Zn(tanm)_2$) and bis(pyrrolidine-*N*-dithiocarbamato)Zn ($Zn(pdc)_2$) complexes exhibited high hypoglycemic activities when they were orally administered in KKA^y mice (Fig. 5(A)). The OGTT indicated that the blood glucose level attained a peak at 30 min after glucose loading, then reduced gradually, and finally returned to the initial normal level after 120 min in the treated groups; however, it did not recover in the control group (Fig. 5(B)). In addition, HbA_{1c} levels improved (Fig. 5(C)) and serum parameters that are indicative of insulin resistance improved after $Zn(tanm)_2$ and $Zn(pdc)_2$ administration for 25 days. Insulin resistance has been considered to play a central role in the development of a range of metabolic disorders. The control KKA^y mice given the vehicle alone exhibited severe hyperinsulinemia, hyperleptinemia, hypertriglyceridemia, and hypoadiponectinemia. However, the parameters for insulin, leptin, and TG levels in the KKA^y mice that received $Zn(tanm)_2$ and $Zn(pdc)_2$ were significantly reduced as compared to those of the control KKA^y mice, suggesting that the two complexes improve insulin resistance. Hypoadiponectinemia induced by visceral fat accumulation is known to become a strong risk factor not only for DM and hypertension but also atherosclerosis and cardiac events. Thus, hyposecretion of defensive adipocytokines such as adiponectin might be the major mechanism of lifestyle-related diseases, including DM, hyperlipidemia, and hypertension, comprising the

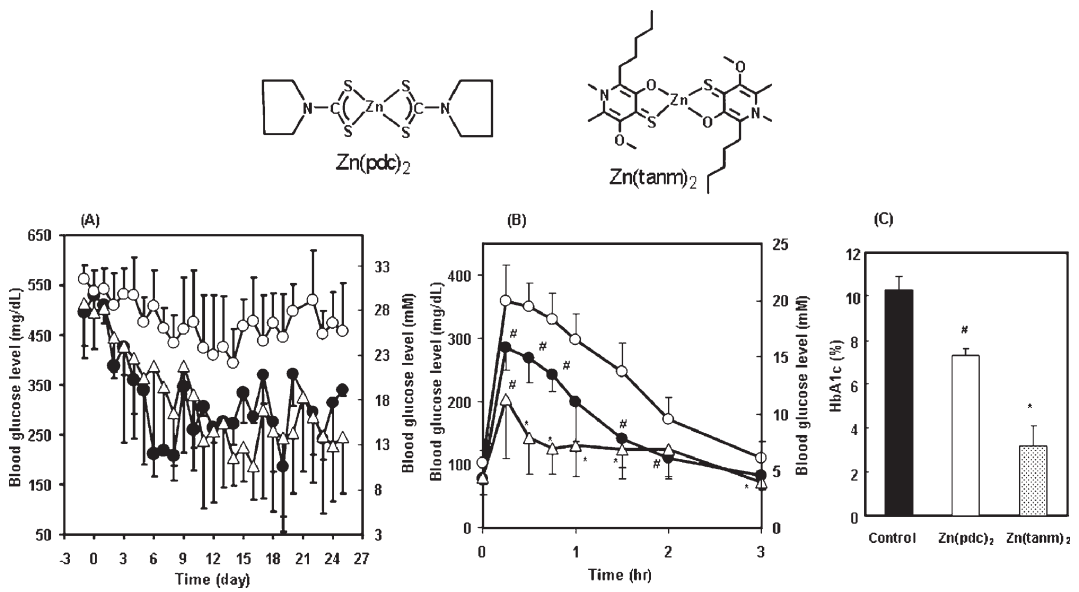


Fig. 5 Effects of $Zn(tanm)_2$ and $Zn(pdc)_2$ on blood glucose, glucose tolerance, and glycated hemoglobin. (A) Changes in blood glucose levels (dose: $Zn(tanm)_2$ (△): 15 mg Zn kg^{-1} BW; $Zn(pdc)_2$ (●): 10 mg Zn kg^{-1} BW for the first 10 days and then 10 or 15 mg Zn kg^{-1} BW for the next 15 days), (B) changes in blood glucose levels during oral glucose tolerance test (OGTT), and (C) HbA_{1c} levels. Data are expressed as the mean values ± SD for 4–6 mice. Significance: $^{\#}p < 0.05$ and $^{*}p < 0.01$ *vs.* control KKA^y mice.

so-called metabolic syndrome. Since the oral administration of $Zn(tanm)_2$ and $Zn(pdc)_2$ complexes normalizes the depressed plasma adiponectin levels in KKA^y mice, these complexes may contribute to the treatment of DM and metabolic syndrome in humans.

There are few studies of Zn supplementation in diabetic patients proposing anti-oxidative effects of Zn. It has been reported that Zn shows a suppressive effect on NF-κB activation in the pancreas, a decreasing effect on plasma thiobarbituric acid reactive substances, and a decreasing effect on the expression of inducible nitric oxide synthase (iNOS).[32]

5. Action mechanism of metal complexes with anti-diabetic and anti-metabolic syndrome activities

It is now established that vanadyl complexes exhibit anti-diabetic effects in experimental animals, and several complexes are thus potential candidates for oral therapy for both type 1 and 2 DM. Despite many studies during the past decade, the action mechanism by which vanadyl compounds mediate *in vivo* anti-diabetic effects are not well understood, and whether or not vanadyl compounds directly mimic or enhance insulin effects is still under investigation. Several studies have shown that vanadyl compounds mimic most of the physiological actions of insulin *in vitro*. However, these *in vitro* insulinomimetic effects of vanadyl compounds may not have therapeutic relevance because these were observed at high doses that are not usually achieved *in vivo*. In order to reveal the mechanism by which vanadyl compounds exhibit anti-diabetic activity, the mode of action of vanadyl compounds has previously been examined by many researchers, and important data have been accumulated with regard to the inhibition of protein tyrosine phosphatase (PTP1B) activity.[33] This activity is involved in the activations of the insulin receptor tyrosine kinase, the cytosolic nonreceptor tyrosine kinase, direct phosphorylation of insulin receptor substrate 1 (IRS1), and the activation of phosphatidylinositol 3 kinase (PI3K), thereby leading to glucose transporter 4 (GLUT4) translocation to the cell membrane. Accordingly, recent research suggests that the suppression of hepatic glucose output through the inhibition of key gluconeogenic enzymes may play an important role in mediating the glucoregulatory effects of vanadyl compounds.[24] However, the results obtained are sometimes controversial; in addition, there have been few reports dealing with the comprehensive mode of action of vanadyl complexes. In a recent study, the molecular mechanism of vanadyl complexes was examined, and $VO(3mpa)_2$, $VO(alx)_2$, and [*meso*-tetrakis(4-sulfonatophenyl)-porphyrinato]oxovanadium(IV) (VO(tpps)) were revealed to affect the tyrosine phosphorylation of IRβ and IRS, leading to the activation of phosphatidylinositol 3-kinase-Akt (PI3k-Akt) signaling and translocation of GLUT4 to the plasma membrane.[34] Furthermore, it was examined whether the $VO(alx)_2$ complex contributes to both the activation of the insulin signaling cascade that activates GLUT4 translocation and the regulation of the forkhead box O1 (FOXO1) transcription factor that controls the gene transcription of glucogenesis genes. The critical functions of the $VO(alx)_2$ complex have been revealed to involve not only the activation of PI3k-Akt signaling through the enhancement of tyrosine phosphorylation of IRβ and IRS, which in turn transmits the signal to activate GLUT4 translocation, but also the regulation of the DNA binding activity of the FOXO1 transcription factor.[34]

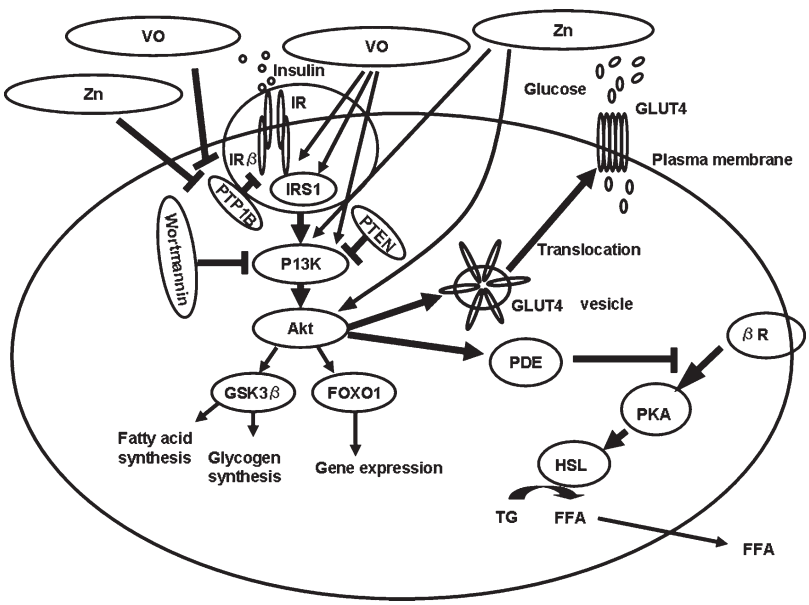


Fig. 6 A possible mechanism for the anti-diabetic vanadium and zinc complexes. The insulin receptor (IR) is a tyrosine kinase that undergoes autophosphorylation, and catalyses the phosphorylation of cellular protein IRS1. Upon tyrosine phosphorylation, the protein activates PI3K, which in turn activates Akt. Thus, GLUT4 is activated and translocated to the cell membrane, leading to the uptake of glucose into the cell. Potential action sites of vanadyl (VO) and Zn complexes are indicated by two signs (inhibition: ⊣ or activation: →). Abbreviations in the figure are as follows. FOXO1: forkhead bOX-containing protein, O sub-family 1; PTEN: phosphatase and tensin homolog detected on chromosome ten; GSK3β: glycogen synthase kinase 3β; PKA: protein kinase A; HSL: hormone-sensitive lipase.

On the other hand, the majority of the studies on the potential protective effect of Zn in the development of DM have been performed in animal models of DM. Zn is a known antioxidant in the immune system. It was tested whether or not an increase in dietary Zn can prevent the onset of type 1 DM by blocking NF-κB (one of the gene transcription factors) activation in the pancreas. The results showed that high Zn intake significantly reduced the severity of type 1 DM in alloxan- and STZ-induced diabetic animal models. Zn supplementation also inhibited NF-κB activation and decreased the expression of iNOS, which is a downstream target gene of NF-κB.[32] Thus, Zn supplementation was concluded to significantly inhibit the development of type 1 DM. In addition, the effects of dietary Zn deficiency and Zn supplementation on hyperglycemic control in db/db mice were compared. Dietary Zn supplementation attenuated hyperglycemia and hyperinsulinemia in db/db mice, suggesting the role of Zn in the pancreatic function and the peripheral tissue glucose uptake. Zn appears to play a role in modulating insulin receptor tyrosine kinase activity in the skeletal muscle of a genetic type 2 DM model mouse. Many researchers have reported insulinomimetic and anti-diabetic activity of Zn. For example, it was reported that the effects of Zn on both glucose oxidation and lipolysis stimulation are inhibited by extracellular catalase, which results in H_2O_2 generation. In addition, Zn was observed to stimulate both lipogenesis and glucose transport in adipocytes, and it was found to increase the phosphorylation of tyrosine in the β-subunit of the insulin receptor and serine-473 in Akt in adipocytes. Furthermore, it was shown that the enzymatic activity of PTP1B is reversibly inhibited by Zn.[35] These results indicate that Zn affects carbohydrate metabolism through the insulin receptor PTP1B and other related proteins. However, the true role of Zn that affects carbohydrate metabolism has not been clarified. Furthermore, there have been few reports dealing with the action mechanisms of Zn complexes. In recent years, the action mechanism of Zn complexes has been examined in isolated rat adipocytes or cultured 3T3-L1 cells, and it has been revealed that Zn complexes, $Zn(pa)_2$ and $Zn(mal)_2$, strongly act on GLUT4 translocation and phosphodiesterase (PDE). Following the study, the molecular mechanism of the $Zn(opt)_2$ complex was examined and it was found that the complex exhibits anti-diabetic activities by activating the insulin signaling cascade through Akt/protein kinase B (Akt/PKB) phosphorylation.[36]

In conclusion, the following results have been obtained: vanadyl and Zn complexes inhibit PTP1B and activate phosphatidylinositol 3-kinase-Akt signaling through the enhancement of tyrosine phosphorylation of IRβ and IRS, which in turn transmits the signal to activate GLUT4 translocation (Fig. 6).

6. Conclusion and future aspect

Since 1990 and 2002, a wide variety of orally active anti-diabetic vanadyl and Zn complexes have been proposed, respectively, based on the results of experiments involving isolated adipocytes and diabetic model animals.[5,13] In 2006, nearly 16 years after the initial findings, the first evidence for the real translocation of a glucose transporter, GLUT4, to the cell membrane was obtained when cultured adipocytes were treated with some vanadyl[34] and Zn[36] complexes. Thus, the development of evidence-based anti-diabetic metal complexes was realized in the 21st century. By using this method, bis(3-methylpicolonato)oxovanadium(IV) ($VO(3mpa)_2$) and $VO(alx)_2$ complexes were discovered; these will be candidates for the next-generation of anti-diabetic pharmaceutics.[34] Following these results, $Zn(tanm)_2$ and $Zn(pdc)_2$ (Fig. 5) were also identified to be evidence-based anti-diabetic complexes. In addition, the two complexes were found to treat metabolic syndromes such as insulin resistance, high leptin levels, obesity, and hypertension.[5]

When a compound is developed with the aim of using it in clinical pharmaceutics, the following five points are most important: (1) occurrence of a good lead compound, (2) high pharmacological activity, (3) low toxicity and low side effects, (4) good pharmacokinetic (PK) and pharmacodynamic (PD) features in terms of its effective concentration in the blood, and (5) evidence of pharmacological action mechanism. Among these points, factors (1), (2), and (5) appear to be established in the framework of the research studies described in this review. However, research on (3) and (4) needs to be intensified in the future. In fact, approximately 30% of candidate organic compounds have failed to be developed as clinical pharmaceutics owing to the lack of suitable PK and PD features. For a PK study, real-time metallokinetics, using an *in vivo* blood circulation monitoring-electron spin resonance (BCM-ESR) method, have been proposed in terms of vanadyl species in the blood of live rats that received a single intraperitoneal injection of complexes. This method will be important not only to obtain evidence for the pharmacological activity of the complexes but also to predict new drug design. For determining the toxicity and adverse effects of these complexes, observations of animals for long-term administrations of the target complex over least one or two years are necessary.

Thus, a comprehensive understanding of the above-mentioned five factors in each candidate complex is essential to produce clinically active oral anti-diabetic complexes in the 21st century.

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