Are Vanadium Compounds Drugable? Structures and Effects of Antidiabetic Vanadium Compounds: A Critical Review

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Abstract: Vanadate can be bioequivalent to phosphate and replace it in cellular metabolism. The detection of insulin-like activity has spurred interest in the development of oral anti-diabetic drugs containing vanadium. We collected and evaluated a vast toxicity data set and discussed molecular aspects related to insulin-mimetic effects of vanadium complexes.

Keywords: Vanadium, anti-diabetic, insulin-mimetic, phospho-mimetic, PTP-1B, toxicity, molecular design.

INTRODUCTION

Inorganic phosphate and its biogenic derivatives act as the natural ligand to protein kinases (phosphorylation) and phosphatases (dephosphorylation). Both enzyme families take part in interwoven molecular cascades for intracellular signal transduction when adding or removing high energy phosphate groups on proteins, i.e. metabolic regulation by activation or inactivation. Such enzymatic processes occur in intracellular signal transduction on insulin coupling at its cell membrane receptor, (Fig. **1**).

Certain vanadium complexes adopt not only the same transition state trigonal bipyramidal geometry as phosphate in the phosphate ester hydrolysis reaction [1], but also the tetrahedral structure and atomic charge distribution of the phosphate ion that explains why vanadate is bioequivalent to phosphate, (Fig. **2**).

They also imitate (mimic) phosphate-like physico-chemical behavior under certain chelating conditions [2]. Their ligand activities at phosphate binding sites (phosphatases, kinases) are the key to understand a great plethora of cellular effects. Crans proposed a multi-target mechanism of action related to what is referred to as insulin-mimetic (insulin-like) effect, because vanadium compounds act in different modes of action, just like insulin does not only lower blood sugar [3]. But vanadium interacts with many more molecular targets what explains undesired effects [4]. Logically, dissociation of toxic and blood glucose lowering effects of vanadium is the major concern to develop specific insulin-mimetic drugs and not just unspecific phospho-mimetics.

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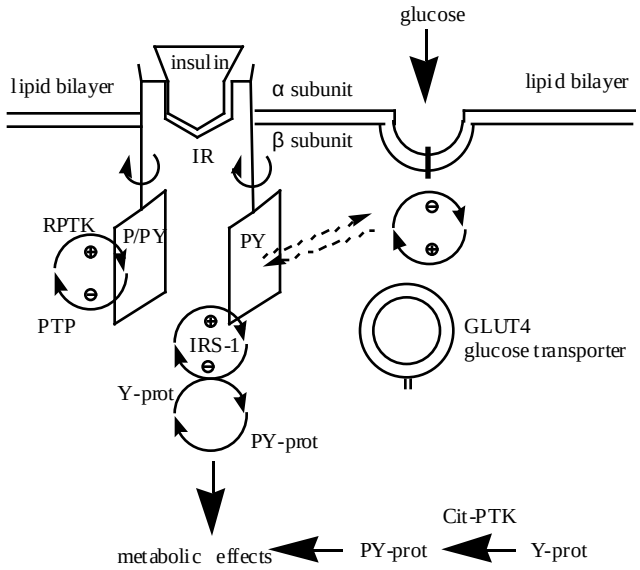

Fig. (1). Initial steps in the insulin signal transduction at the cellular level. Insulin binding (on top) to the extracellular α-subunit of the transmembranal insulin receptor (IR) leads to a conformational change in the intracellular β-subunit and promotion of the autophosphorilation of tyrosine residues, Y + P = PY (below). The receptor protein tyrosine kinase (RPTK) is a positive regulator of the signal transduction, while protein tyrosine phosphatases (PTP) are negative ones. The IR acts as a tyrosine kinase transferring phosphate to different receptor substrates, here IRS-1, which propagates the insulin signaling through phosphate transfer to citoplasmatic proteins (Y-prot), giving place to metabolic effects. In the process, insulin increase glucose transport in fat and muscle cells by stimulating the translocation of the transporter GLUT4 from intracellular sites to the plasma membrane and promoting its exocytosis. Scheme modified after Saltiel, A.R. and Khan, C.R. *Nature*, **2001**, *414*, **799**.

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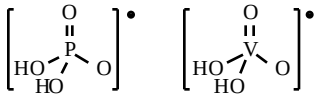

Fig. (2). Geometries of phosphate, $H_2PO_4^-$, (left) and vanadate, $H_2VO_4^-$, (right) anions. The pKa values for $H_2PO_4^-$ and $H_2VO_4^-$ are 7.2 and 8.1, resp.. The monoanionic forms prevail over the dianionic species at physiological pH. In the case of vanadate, however, the concentration should be below 0.3 mM to preclude polymerization. Reference [6] page 519 and page 984.

To start with, we recommend the reading of five cardinal works dealing with molecular aspects of vanadium compounds with insulin-like activity (Table **1**). We concentrate on relevant topics: Chemistry and stability, pharmacology, toxicity, specificity and selectivity concerning antidiabetic effects of vanadium complexes.

AQUEOUS CHEMISTRY

Vanadium, atomic number 23, is a first row transition metal that shows a wide range of oxidation states in monomeric, oligomeric and polymeric species in solution [5]. Vanadium is a heavy metal that occurs in forms of two natural isotopes, ^{50}V and ^{51}V, the latter being the naturally occurring radioisotope. It exists in oxidation states of –I, 0, +II, +III, +IV and +V; the latter two are stable solution structures at physiological pH: vanadyl (+IV) and vanadate (+V). Their geometries depend upon the ligand number: tetrahedral with 4 ligands, trigonal bipyramidal: 5, octahedral: 6, pentagonal bipyramidal: 7 and dodecahedral: 8 [9], (Fig. **3**).

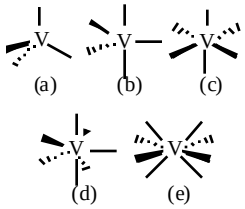

Fig. (3). Different geometries in vanadium inorganic and organic compounds. (a) tetrahedral, (b) trigonal bipyramidal, (c) octahedral, (d) pentagonal bipyramidal, and (e) dodecahedral.

Vanadyl: Aqueous acidic solutions of vanadium(IV) sulfate, $VOSO_4$, contains hydrated vanadyl cation, $VO(H_2O)_5^{2+}$, also called **oxovanadium(IV)** or just vanadyl, abbreviated VO^{2+}. This dinuclear specie forms stable compounds with F, Cl, O and N donor ligands. Typically, such vanadyl complexes show a bluish to greenish color and can be cationic, neutral or even anionic. Except for water (6-coordinated), they use to be 5-coordinated in organic solvents, i.e. the geometry is almost invariably square pyramidal. $VO(acac)_2$ is the archetype of this geometry, but trigonal bipyramidal geometry is also possible in compounds with high volume ligands. At physiological pH, vanadyl forms oligomeric and polymeric species, however, complexation prevents the formation of polymeric solids [5].

Vanadate: Vanadium(V) also forms stable compounds with the same atom-donor ligands as vanadium(IV), but has a greater affinity for O-donors. The color of salt V_2O_5 is orange to yellow due to the **dioxovanadium(V) ion**, $[VO_2]^+$, and in light alkaline media produces colorless solutions. At higher pH, the prevalent specie is the deprotonated orthovanadate ion, $[VO_4]^{3-}$ while at physiological pH and at concentrations under $1.0x10^{-3}$ M, it predominates the monomeric specie $[H_2VO_4]^-$, independently of the starting salt ($NaVO_3$, NaH_4VO_3, Na_3VO_4, V_2O_5) used to prepare the solution. If H_2O_2 is added to aqueous solutions of $[VO_4]^{3-}$, a series of substituted products is obtained depending on pH. Using Raman and ^{51}V NMR spectroscopy to compare the solutions with compounds of known compositions and structures suggests that the red-brown acidic solutions contain $[VO(O_2)(H_2O)_4]^+$ and that in progressively more alkaline solutions, $[VO(O_2)_2(H_2O)]^-$, $[VO_2(O_2)_2(H_2O)]^{3-}$, $[VO(O_2)_3]^{3-}$ and $[V(O_2)_4]^{3-}$ are formed [5, 57]. The rate of vanadium(V) oligomerization is minimal at concentrations lower than $50x10^{-6}$ M [7].

CHELATE STABILITY

Vanadium compounds are thermodynamically stable but kinetically labile. They undergo competition reactions in solution, exchanging solvent or other potential coordinating molecules available in the media, e.g. $VO(maltolate)_2$ suffers ligand replacement in water. The lifetime of vanadium complexes might be prolonged in physiological fluids, and the interaction with membranes appears to be important in the stabilization of vanadyl complexes [8]. To probe specific target affinity (selectivity) and elimination of undesired enzymatic pathways by reacting fragments, chemical stability of complexed vanadium with organic chelators is crucial. Only stable vanadium complexes avoid ligand replacement by a stronger chelator. Such a natural chelator is ubiquitous citrate, an important constituent of the blood serum, and a very efficient vanadyl binder in a wide pH

Table 1. Suggested Reading

Title	Ref
Antidiabetic vanadium(IV) and zinc(II) complexes	132
The Chemistry and Biochemistry of Vanadium and the Biological Activities Exerted by Vanadium Compounds	147
Vanadium Compounds as Insulin Mimics	148
Insulin-like effects of vanadium: basic and clinical implications	149
Historic perspective and recent developments on the insulin-like actions of vanadium; toward developing vanadium-based drugs for diabetes	150
Vanadium—an element of atypical biological significance	163

range. Certain *in vitro* experiments allow estimation of ligand affinities to vanadate, when based on an already known reference value for the apparent formation constant. Here, with EDTA complex as reference, the calculated decreasing complex stability order was: VO(edta) $1.4x10^4$ > $VO(maltolate)_2$ $1.24x10^3$ > $VO(citrate)_2$ $0.56x10^1$ [7]. However, for VO(edta), another value was assessed later on, which compromises the approximation made with the consequence, that actually citrate complexes are far stronger than organovanadium complexes: VO(edta) $3.80x10^5$ < $VO(maltolate)_2$ $1.05x10^7$ < VO_2dipic^- 4.5×10^8 < $VO(citrate)_2$ $3.16x10^{11}$ < Fe(edta) $1.58x10^{14}$ [3, 9].

Vanadyl: Citrate readily forms ternary complexes when reacting with known insulin-mimetic compounds, as in the case of VO(IV)-maltolate-citrate [10], where the pH-metric speciation measurements and spectral studies unambiguously suggest enhanced ternary complex formation in the pH range 4-8, whereas EPR spectrum of binary VO(IV)-citrate is not observed. Other ternary citrate complexes reported are dinuclear oxovanadium salts: VO(IV)-picolinic acid-citrate and VO(IV)-6-methylpicolinic acid-citrate [11], where the presence of picolinic acid suppresses the exclusive formation of the dinuclear oxovanadium complexes with citrate, which in turn are the predominant species in the binary system in the pH range 3-9. These findings take even more relevance with the fact that other biogenic ligands as oxalic acid, lactic acid and phosphate were inefficient binders towards oxovanadium in the presence of citrate, and that ternary complex formation with citrate involving oxalate, lactate or phosphate is negligible [12].

Vanadate: Vanadium(V) complexes are less labile than vanadium(IV), and they tend to resist the formation of ternary complexes [13]. In order to encounter the formation of ternary complexes, the vanadium(V) organic compounds must be reduced to vanadium(IV), since in this oxidation state, this element can accommodate a citrate ligand in its coordination sphere. Citrate itself could be the reducing agent, as it is well known for a number of metallic salts. Other reducing agents of natural occurrence in plasma are glutathione, cysteine and L-ascorbic acid [14].

PHYSIOLOGICAL TRACE ELEMENT

Whether vanadium is an essential trace element for human life or not, is an unresolved question, since lower concentrations (range: $1x10^{-6}$ M) are well-tolerated and daily intake occurs unavoidably due to its omnipresence in food. Thus, no deficiency syndrome is known so far to assert its need, while gene regulation of MAP kinases, ras, TNF, interleukin among others and biotransformation of glucose or lipids are associated with this element [163]. The average concentration in food is lower than $0.02x10^{-9}$ mol/g, which is very low compared to e.g. iron $350x10^{-9}$ mol/g [15]. The daily estimated intake ranges normally from 0.2 to $1.2x10^{-6}$ M, food being the major source and not drinking water** (< $0.02x10^{-6}$ M) [16].

*Mount Fuji water contains exceptionally vanadium (65 microg/l) and improved blood glucose of rats with DM II at doses of 0.53 microg/kg/day for 12 weeks [161].

PRELIMINARY PHARMACOLOGICAL DATA IN HUMAN BODY

(i) Oral absorption of inorganic salts is very low (<5 %) and almost all is lost with feces.

(ii) Distribution: 90% of circulating vanadium (<$4.0x10^{-8}$ M) is transferrin-bound. Thus, interaction with iron metabolism can be expected.

(iii) Renal elimination: Unlike lead which has a (relative) short half-life (as far as heavy metals are concerned) in human tissue from 30 to 100 days [17], vanadium species dissolved in water have a very short half-life from 20 to 40 hours [16]. Its fast body elimination augments tolerance while lowering acute toxicity.

(iv) Tolerated range: Daily doses of 0.150 g of $VOSO_4$ ($0.9x10^{-3}$ mol/day) already showed efficiency during a 6 week study in humans, Table **2** [18]; and 0.100 g ($0.6x10^{-3}$ mol/day) let to only minor side effects in humans during 4 weeks [16]. Initial clinical studies were performed with doses of 0.02 g/Kg of vanadium per day ($0.04x10^{-3}$ mol/Kg/day) [7].

(v) Oral lethal dose: For V_2O_3 in mice, LD50 0.130 g/Kg ($0.8x10^{-3}$ mol/Kg); for V_2O_5, 0.023 g/Kg ($0.1x10^{-3}$ mol/Kg), what is about ten-fold higher than used above. Beware, bioavailability and susceptibility may vary among species: the parenteral administration of V_2O_5 in guinea pig resulted in a LD50 of 0.025 g/Kg ($0.1x10^{-3}$ mol/Kg), whereas in rabbits only 0.002 g/Kg ($0.01x10^{-3}$ mol/Kg). Still, for many researchers, a promising therapeutic potential exists at doses that do not produce acute toxicity or do it to a limited extent [18-27]. Passive diffusion such as cellular uptake mechanism was identified for vanadate, BMOV and $VO(acac)_2$ [23].

ACUTE TOXICITY

Typical clinical manifestations are: (light) diarrhea, vomiting, abdominal cramps, green tongue, (severe) bronchospasm, neurological and irreversible renal excretion damage.

Daily oral doses up to 0.060 g of $VOSO_4$ or $0.4x10^{-3}$ mol of V (our estimation $5.0x10^{-6}$ to $8.0x10^{-6}$ mol/Kg in humans probably weighing 50 to 80 Kg) are administered to improve muscle performance and structure as a questionable practice of legal sports doping. During 12 weeks a dose of $5.0x10^{-4}$ g/Kg/day ($3.0x10^{-6}$ mol/Kg/day) did not reveal any hematological symptoms in 31 athletes. Probably, a five hundred-fold higher accidental oral exposition (estimated in 10 g) of pentavalent vanadium salt NH_4VO_3 ($85x10^{-3}$ mol of vanadium) was clinically treated with success against gastrointestinal disorder and severe facial paresis [16]. To this regard, the trace element is apparently well-tolerated.

CHRONIC TOXICITY

The rationale behind the deviation towards vanadium-free insulin-mimetic research [28] is most likely to avoid typical heavy metal toxicity (nervous system abnormalities like motor and cognitive disorders, impairment of

Table 2. Period 2004-1999

Experimental conditions	Experimental values	Ref.
Vanadium (V) complexes of composition $VO(O_3N)$. the compounds trigger glucose intake into human and simian virus modified mice fibroblasts, in several cases at a higher level than insulin.	fibroblast cell cultures; C (V)= 0.1 mM. Not toxicity reported	153
The uptake of $VO(acetylacetonate)_2$ and $VO(maltolate)_2$ by human erythrocytes showed intracellular vanadium level higher than $NaVO_3$ and the membrane transport of these two vanadyl complexes was presumed to be via the passive diffusion mechanism.	human erythrocyte membrane vesicles; 375 mM oxovanadium compound	8
vanadium(IV and V) compounds tested in transformed mice fibroblasts (cell line SV 3T3), with respect to their short-term cell toxicity (up to 36 hours) and their ability to stimulate glucose uptake by cells. Complexes containing thio functional ligands are somewhat more toxic than others.	Toxic at c(V)≥ 1 mM; Non-toxic at c(V)≤ 0.01 mM; V(V) less toxic than V(IV).	19
Toxicity of V varies with the nature of the compound, it is being toxic as both as cation and as an anion. Toxicity increases as valency increases, V 5+ being the most toxic. V pentoxide is more soluble and more toxic than the trioxide or dioxide. Toxic effects of V include adverse effects on the respiratory system, central nervous system, digestive system, kidneys and skin. Acute and chronic V toxicity largely result from inhalation exposure; acute toxicity depends upon the concentration of the V, the sensitivity of the individual and the properties of the specific V compound. The more soluble salts of V pentoxide have a more rapid action than the other V oxides. V chloride is more rapidly toxic than other V compounds.	Clinical features of mild toxicity include rhinitis, with a profuse and often bloody discharge, sneezing, an itching and burning sensation in the throat, general weakness and exhaustion. Conjunctivitis is frequently observed.	43
Vanadyl sulphate reacts with the peroxy acid oxidant $KHSO_5$ to produce guanine-selective oxidation of a 167 bp restriction fragment of DNA.Vanadyl sulphate is able to oxidize DNA especifically at guanine residues.	Vanadyl sulphate 30 μM, incubated with fragments of DNA	151
Exposition of rat renal brush border membrane vesicles (BBMV) to vanadium pentoxide and examination of their citrate uptake characteristics. The preincubation of BBMV with 1 mM V_2O_5 for 8 h significantly inhibited citrate uptake compared with that of BBMV without V_2O_5 preincubation. These findings indicate that the preincubation of BBMV with vanadium pentoxide results in a time-dependent inhibition of citrate uptake.	1 mM V_2O_5 for 8 h vanadium exposure might contribute to nephrotoxicity	152
(4-hydroxypyridine-2,6-dicarboxylato) oxovanadate (V). The growth of myoblast cells (L6) was inhibited by vanadium(V) complex. Yeast growth (pH range from 3.0 to 7.0) was employed as an adjunct cell model. The effect of the vanadium(V) complex on inhibition of yeast cell growth was found to be pH dependent.	Administered to cell culture, yeast and streptozotocin (STZ)-induced diabetic rats; 1.6 to 6.2 mM. Low toxicity	21
25 bis(cyclopentadienyl)vanadium(IV) and 14 oxovanadium(IV) compounds. synthesized and evaluated for anticancer activity, bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) (metvan) was identified as the most promising multitargeted anticancer vanadium complex with apoptosis-inducing activity. Metvan shows favorable pharmacokinetics in mice. Therapeutic plasma concentrations > or = 5 _μM.	12.5-50 mg/kg not acute or sub-acute toxicity; highly cytotoxic against human cancer cells, a single injection of 10 mg/kg of metvanvandate	78
Treatment significantly improved glycemic control; Diabetics had an increased rate of endogenous glucose production compared with nondiabetic controls; The glucose-lowering effect of $VOSO_4$ correlated well with the reduction in EGP, but not with insulin-mediated glucose disposal, suggesting that liver, rather than muscle, is the primary target of $VOSO_4$ action at therapeutic doses in T2DM.	11 type 2 diabetic patients were treated with $VOSO_4$ at a 150 mg/day dose for 6 weeks.	17
vanadate induces p53 activation mainly through H_2O_2 generation, and this activation is required for vanadate-induced apoptosis. Mitochondrial damage, apoptosis, ROS	mouse epidermal JB6 cells treated with vanadate	50
Under vanadate stimulation A546 cells generated OH radical, H_2O_2, superoxide.	human lung epithelial cell line (A549) treated with vanadate	90
vanadyl acetylacetonate, vanadyl 3-ethylacetylacetonate, bis(maltolato)oxovanadium, and vanadyl sulphate. Organic vanadium induced a faster and larger fall in glycemia than inorganic. Glucosuria and tolerance to a glucose load were improved accordingly. Vanadyl acetylacetonate rats exhibited the highest levels of plasma or tissue vanadium, most likely due to a greater intestinal absorption. However, it retained its potency when given as a single i.p. injection to diabetic rats. No relationship was found between plasma or tissue vanadium levels and glucose metabolism; Thus, these data suggest that differences in potency between compounds are due to differences in their insulin-like properties. There was no marked toxicity observed on hepatic or renal function.	non-ketotic, streptozotocin-diabetic rats; oral administration 125 mg V/L in drinking fluids for 3 months. No toxicity on hepatic or renal function; diarrhoea in 50% rats chronically treated with vanadyl sulphate	23
The most cytotoxic analogue, bpV[4,7-dimethyl-1,10-phenanthroline-bisperoxo-oxo-vanadium (Me_2Phen)], shows submicromolar IC50s against a panel of cell lines and inhibited tumor growth by 80% in mice. These results demonstrate that bpVs may have significant antineoplastic activity. In addition, they are in vitro and in vivo inhibitors of phosphatases including Cdc25A, suggesting that phosphatases may be appropriate targets for novel antineoplastic agents and that further development of these agents, targeting them to specific phosphatases such as CDC25A, may lead to novel agents with enhanced antineoplastic activity.	Cytotoxicity of Bisperoxovanadium (bpV) compounds was examined in 28 cancer cell lines and in vivo efficacy was examined in a DA3 murine mammary carcinoma model	80
Only the vanadium(IV)-containing metallocenes exhibited significant cytotoxicity against Tera-2 and Ntera-2 cells and induced apoptosis within 24 h. Vanadocenes with dithiocyanate [$VCp_2(SCN)_2 \cdot 0.5\ H_2O$] and diselenocyanate [$VCp_2(NCSe)_2$] as ancillary ligands were identified as the most potent cytotoxic compounds. Vanadocenes, especially the lead compound $VCp_2(NCSe)_2$, may be useful in the treatment of testicular cancer	five metallocene dichlorides of vanadium, titanium, zirconium, molybdenum, and hafnium, and 19 vanadocene complexes were tested against the human testicular cancer cell lines Tera-2 and Ntera-2	81
After 6 days of intraperitoneal administration of both $VO(acac)_2$ and melatonin alloxan-diabetic rabbits, vanadium-induced elevated serum creatinine and urea levels were decreased, indicating the beneficial effect of melatonin on diabetes and vanadium-induced nephrotoxicity in rabbits	i.p. administration of $VO(acac)_2$ and mellatonin to diabetic rabbits for 6 days	42

Table 3. Period 1998-1990

Experimental conditions	Experimental values	Ref.
A significant decrease in the pregnancy rate was observed at 60 and 80 mg/kg per day of sodium metavanadate; however, vanadium would not cause any adverse effect on fertility or testicular function in male mice at the concentrations usually ingested by humans through the diet and drinking water.	Male Swiss mice exposed to sodium metavanadate at doses of 0, 20, 40, 60, and 80 mg/kg per day given in the drinking water for 64 days	46
Vanadate treatment was found to be toxic during diabetic pregnancy, causing death to 45% of the test animals; maternal blood vanadium had a negative effect on fetal development, markedly reducing the number of live fetuses per pregnancy	Sprague Dawley rats treated with sodium vanadate; 62.5 or 125 mg/kg bw/day) throughout gestation	48
V_2O_5 treatment resulted in a decrease in fertility rate, implantations, live fetuses, and fetal weight, and an increase in the number of resorptions/dam. Sperm count, motility, and morphology were impaired with the advancement of treatment. It is concluded that vanadium pentoxide was a reprotoxic and genotoxic agent in mice	testis cells treated with V_2O_5	45
Mild gastrointestinal intolerance and diarrhoea was reported in both groups, including vomiting in one patient, which limited the total daily dose to 75 mg. No biochemical evidence of toxicity was detected by screening, which included electrolytes, blood urea nitrogen, creatinine, liver function studies, thyroid functions, urine analysis and a complete blood count.	5 IDDM and 5 NIDDM patients, studied before and after 2 weeks of oral sodium metavanadate ($NaVO_3$; 125 mg/day)	25
Vanadyl sulphate was associated with gastrointestinal side effects in six of eight patients during the first week, but was well tolerated after that. Modest reductions of fasting plasma glucose and hepatic insulin resistance was achieved.	100 mg vanadyl sulphate per day to eight NIDDM patients, for 4 weeks	26
Vanadyl sulphate, administered to six non-insulin dependant diabetic (NIDDM) patients in a single-blind placebo controlled trial, resulted in mild gastrointestinal symptoms; nausea, mild diarrhoea and abdominal pain.	Vanadyl sulphate administered at six non-insulin dependant diabetic patients; 100 mg/day for three weeks	111
Myocardial contractile force decreases, in spite of the fact that the content of calcium is considerably higher than that in the control group. These results also show a toxic effect of vanadate on the myocardium, probably due to large intracellular accumulation of calcium and cell damage.	Intragastric administration of Na_3VO_4 (0.03 mmol/kg/ daily) to rats	51
Plasma and tissue concentrations of vanadium were distributed as follows: bone > kidney > testis > liver > pancreas > plasma > brain. Vanadium was retained in these organs at 16 weeks following vanadyl withdrawal while the plasma levels were beneath detection limits. Vanadyl sulphate at antidiabetic doses is not significantly toxic to rats following a one-year administration in drinking water, but vanadium may be retained in various organs for months after cessation of treatment.	Streptozotocin-diabetic and non-diabetic rats were given vanadyl sulphate in drinking water at concentrations of 0.5-1.5 mg/ml for one year	53
All animals exposed to vanadate had increased systolic and diastolic blood pressure. This effect was not dose dependent and heart rate and cardiac inotropism were not affected. The following changes was observed: (a) decreased Na, + K(+)-ATPase activity in the distal tubules of nephrons; (b) increased urinary excretion of potassium; (c) increase in plasma renin activity and urinary kallikrein, kininase I, and kininase II activities; (d) increased plasma aldosterone (only in the rats treated with 10 ppm of vanadium).	1, 10, or 40 μg/ml of sodium metavanadate, for 6 or 7 months administered male Sprague-Dawley rats	52
The use of titanium and titanium-6% aluminum-4% vanadium alloy for dental and orthopedic implants has increased in the last decade. This study examined the uptake of salts or fretting corrosion products. Titanium was not observed to be toxic to the cells. Vanadium was toxic at levels greater than 10 μg/ml	10 μg/ml	24
$VOSO_4$ treatment partially or totally corrected some of the alterations associated with the diabetic state (hyperglycaemia, polydipsia, polyphagia, high cholesterol and triglycerides levels) and did not produce any changes in various plasma or blood cell parameters which were not previously altered by diabetes. Measurement of vanadium levels indicated that tissues accumulated vanadium in the following order of concentrations: bone > kidney > spleen > liver > lung >= muscle > blood.	0.25, 0.5, 0.75 or 1 mg of $VOSO_4$ administered in drinking water to STZ-diabetic rats	154

gastrointestinal tract and renal, hepatic, cardiovascular, respiratory, immune and reproductive systems) as non-dissociable intrinsic effects. A great plethora of side effects can be expected from the phosphate imitation, (Fig. **2**), if target selectivity is missing: activate cellular transcription factors [29, 30], apoptosis [31], phosphorylation by kinases, known to be vital for cell cycle in mammals [32] and expression of regulatory genes necessary for initiating DNA replication [33, 34]. Scripted side effects to vanadium intake are also found in [35] and [36]. A viva discussion on the risks took place between two opposing groups [37–41]. Here, we summarize the data about doses, toxic effects, ranges and concentrations found in experimental work (Tables **2-4**).

The analysis of Tables **2-4** suggest that vanadium toxicity depends on specific chemical form, oxidation state, administration route, period and doses, as well as type of studied organism. The concentration limit for which vanadium compounds become toxic also depends on the type of coordination environment. As a general rule, concentrations below $0.01x10^{-3}$ M are estimated to be safe and still are able to maintain the biological activity, whereas those above $1.0x10^{-3}$ M are expected to be toxic for chronic use.

Patterns of vanadium toxic strength may be resumed as follows: soluble > insoluble forms; parenteral > pulmonary >> oral administration; inorganic salts > organic vanadium

Table 4. Period 1989 and Before

Experimental conditions	Experimental values	Ref.
In doses that controlled blood glucose in diabetic animals for a 1-year no biochemical, histological or functional changes were noted	vanadyl sulphate administered to control and STZ diabetic rats for 1 year	155
Disturbances of the gastrointestinal tract include vomiting and diarrhoea, together with bronchospasm, this points to a response of the smooth muscle to vanadium exposure. Cutaneous manifestations: rashes and eczema. Bronchitis and ronochopneumonia are features of severe toxic effects. Other symptoms includes headache, vomiting, diarrhoea, palpitations, sweating and general weakness. Disorders of the nervous system include severe neurotic states and tremor of the fingers and hands. Kidney damage, highlighted by grave dystrophic changes in the epithelium of the convoluted tubules and disturbed tubular excretion, occurs immediately after exposure to low vanadium doses, in cases of both acute and chronic toxicity and is not reversible even if exposure is discontinued	Acute human vanadium exposure to vanadium pentoxide; dust concentrations range from 0.1 to 30 mg/m^3, and concentrations of 0.5–5 mg/m^3	156
20 animals per dose group and control animals received injections of phosphate buffered saline alone. Although no significant maternal toxicity was apparent at any of the dose levels, skeletal anomalies were observed in the offspring. In spite of these anomalies, it was concluded that the low incidence and lack of dose-dependent response would not allow a definite correlation of teratogenicity with ammonium vanadate exposure	pregnant hamsters received ammonium vanadate (0.47, 1.88 and 3.75 mg/kg bw/day) by *i.p.* injection from days 5 through to 10 of gestation	47
Treatment of the females was continued during the periods of gestation and lactation. Although no significant effects on fertility, reproduction and parturition were observed, the development of the offspring (body weight, body length and tail length) was significantly decreased from birth and during the lactation period, at all dose levels. Sodium metavanadate was neither embrytolethal, or teratogenic in rats at doses of 20 mg/kg bw/day or lower when it was orally administered, on days 6 to 14 of gestation	20 adult male rats were administered metavanadate; 0, 5, 10 and 20 mg/kg bw/day by oral gavage for 60 days; mating with females that received the same vanadate doses for 14 days just prior to mating	49
Not increase the incidence of spontaneous tumours	Life-time studies in mice given 5 µg/ml vanadyl sulphate in drinking water	157
Cell viability after a 20-hr exposure was reduced by 50% at approximately 13 µg V/ml as V_2O_5, 21 µg V/ml as V_2O_3, and 33 µg V/ml as VO_2; Cytotoxicity was determined to be directly related to solubility in the order $V_2O_5 > V_2O_3 > VO_2$; When V_2O_5 was dissolved in media prior to exposure of cells, dissolved V_2O_5 at concentrations as high as 50 µg V/ml produced only small changes in the specific activities of lysozyme or –glucuronidase; acid phosphatase was 70% inhibited by dissolved V_2O_5 at 1 µg V/ml. Exposure to vanadium oxides may alter alveolar macrophage integrity and function to the detriment of pulmonary defense	rabbit alveolar macrophages were exposed to particulate forms of vanadium pentoxide (V_2O_5), vanadium trioxide (V_2O_3), or vanadium dioxide (VO_2)	44
Non toxic daily intake of vanadyl sulphate by food, multivitamins and drinking water; *in vivo* studies in humans, side effects with diarrhea and nausea	vanadyl sulphate < 1 ng /g food; ca. 13 µg /24 h food;10 µg multivitamines; 1-30 µg /L water; 100 mg /24 h	20
Evaluation of the potential of various metallic compounds to induce lung adenomas in strain A mice. In one test group, The incidence of lung tumors was not significantly different from that of the controls	mice were given 24 i.p. injections of vanadium (III) 2,4-pentanedione (total dose, 120, 60, or 24 mg/kg) over a 30-week period	158
Murine mammary carcinogenesis was blocked by the feeding of a purified diet formulation supplemented with 25 ppm. vanadyl(IV) sulfate during the post initiation stages of the neoplastic process. Treatment reduced both cancer incidence and the average number of cancers per rat and prolonged the median cancer-free time without inhibiting the overall growth of the animals. Vanadyl(IV) sulphate appears to be an effective non-toxic agent for the chemoprevention of experimental breast cancer in the rat	vanadyl(IV) sulfate 25 ppm. administered in diet formulation to rats induced to carcinogenesis by 1-methyl-1- nitrosourea	82

compounds; vanadium(V) organic compounds > vanadium(IV) organic compounds.

In the case of vanadium organic compounds, the toxicity also depends on the coordination environment of vanadium: the compounds with a combination of donor atoms NN, OO, or NO are less toxic than compounds with donor atoms NS, OS, or SS, irrespective of the vanadium oxidation state. In general, the use of organic ligands seems to decrease the toxic effects. The toxicity tends to decrease as the valence decreases: so, pentavalent compounds are the most toxic ones in aqueous solutions. Since the state of oxidation is reversible and sensitive to pH, acidification usually reduces toxicity by lowering the oxidation state to more stable ones.

Another aspect related to the vanadium therapy is that the co-administration of complexing agents may reduce the potentially toxic effects: administration of tiron did not diminish the reduction in plasma glucose levels caused by vanadate, but its accumulation in kidney, bone, liver, heart and muscle was significantly decreased in the tiron-treated rats groups [42]. In the same sense, melatonin also reduces toxic effects of administered vanadium [43]. Concerning aqueous dissolution, some reports point out that toxicity is related with solubility [44, 45], but this effect seems to affect only the oral absorption of vanadium, because LD50 data does not correlate with the extent of the solubility (Table **5**).

Table 5. Solubility and LD50 Data for Most Common Vanadium Salts

Name(s) of Vanadium Salt	Formula	Solubility in water g/L 20 °C [a]	LD50 mouse mg/Kg
Vanadium pentoxide (vanadium(V) oxide, divanadium pentoxide)	V_2O_5	8	23 [b]
Ammonium vanadate (ammonium metavanadate, vanadic acid ammonium salt)	NH_4VO_3	58	10 [b]
Vanadium sulfate (vanadyl sulphate, vanadium(IV) sulphate oxide)	$VOSO_4$	soluble	10 [b]
Sodium vanadate (sodium metavanadate, vanadic acid sodium salt)	$NaVO_3$	211 (25 °C) 388 (75 °C)	75 [c]
Sodium orthovanadate (sodium tetraoxovanadate)	Na_3VO_4	slightly soluble	300 [d]

[a]Agency for Toxic Substances and Disease Registry, *ATSDR's Toxicological Profiles: Vanadium*. Boca Raton, Florida: Lewis Publishers, CRC Press, Inc., **1997**.
[b]WHO expert committee, World Health Organ. *Tech. Rep. Ser*. **1988**.
[c] Material Safety Data, ACROS Organics.
[d]CHEMICON International, a Division of Serologicals Corporation, Cat. No. 2140.

ACCUMULATION

Vanadium tissue accumulation represents the major concern about vanadium use in long-term administration [38]. *In vitro* cytotoxicity can be related to vanadate uptake, subsequent elevation of intracellular reactive oxygen species (ROS) and their cellular accumulation [23]. Related with tissue accumulation, there exists a developmental risk of partial crossing of blood brain barrier, blood testes and placental barriers, and also maternal toxicity with embryotoxicity, fetotoxicity and teratogenicity in mice administered with >0.050 g/Kg/day. Therefore, vanadium compounds should not be used during the periods of pregnancy and lactation [46-50]. Other toxic effects that could derive from the chronic use of vanadium are the generation of ROS and apoptosis [51], also heart dysfunction [52, 53]. However, other authors think that the accumulation in tissues is not necessarily a toxic effect, since there must be a pathological change in order to be so classified [39]. It is noticeable that vanadium is stored in various organs with long half-lives in the body and its prolonged presence may potentially maintain some anti-diabetic activity [54, 55]. Vanadium inorganic salts are not well absorbed by human organism, roughly 5% of the ingested mass and only in certain conditions can exceed 10% [56]. Most ingested vanadium is apparently unabsorbed and is thus excreted *via* the feces [57]. Once incorporated, it tends to accumulate like phosphate. Because of the chemical relatedness, retention in bone mass is predictable. Accumulation can be related with other phosphate dependent steps of metabolism, e.g. in liver and pancreas, and in places of high energy consumption as heart and brain. It has been found that the distribution of vanadium in body tissues after oral and intraperitoneal administration follows the order: bone > kidney > liver > spleen > heart > testes > lung > pancreas > brain after 24 h [58]. There is evidence that vanadyl interacts with bone mineral compound hydroxyapatite [59], but it is now known that vanadyl ions did not incorporate into the apatitic lattice and could therefore be expected to interact strongly with the surrounding aqueous bilayer [60], this corresponds well with the observation that vanadium is deposited in bone and it does not appear to affect bone strength or architecture [61].

On the other hand, vanadium organic complexes accumulation follows a pattern that is not quite different from inorganic salts. The principal sites of accumulation are bone, kidney and liver, however, the absorption of these compounds is highly improved, their retention time in plasma is also longer and their biological effects enhanced, which in turn is not merely due to the increased absorption, but depends strongly on the chemical structure [62]. This means that distribution and accumulation is related with structure. For example, ^{48}V marked BMOV showed a distribution pattern of bone > kidney > liver in a 24 h trial, and the concentrations detected were 3 times higher than the same experiment using vanadyl sulfate [42].

ENZYMES AS SPECIFIC TARGETS FOR VANADIUM ACTIVITY

Phosphate-like ligand-enzyme activity of vanadium: it is the key interaction for its insulin-like activity, but also the origin of some side effects. Mechanisms of the insulin-mimetic effects of vanadium compounds are not yet completely elucidated: Although well-documented to inhibit protein tyrosine phosphatase family enzymes (PTPs) [63-65], vanadium compounds have been suggested to (i) directly activate tyrosine kinases [65], (ii) inhibit glucose-6-phosphatase (G-6-Pase) [66, 67], (iii) inhibit protein degradation [68], (iv) alter phosphoinositide metabolism [69, 70] and (v) bind to a variety of small molecules, such as ADP, GDP and NADH [71, 72], all of which may influence cell signaling°.

(i) *In vivo* insulin-mimetic effects of vanadium are thought to involve non-insulin-receptor-dependent tyrosine kinases, the stimulation of the Na^+ /K^+-ATPase as well as the rise in cell K^+ which could stimulate protein synthesis [73].

(ii) Chronic hyperglycemia is shown to stimulate endogenous glucose production (EGP) by up-regulating the G-6-Pase complex as the final step involved in the release of glucose by the liver. Vanadyl sulfate treatment, in DM II patients, improves glycemic control by reducing basal EGP and enhancing skeletal muscle insulin sensitivity [66]. Also BMOV when administered to diabetic rats, inhibits hepatic phosphoenolpyruvate carboxykinase (PEPCK) and G-6-Pase mRNA expression [67].

°Vanadyl complexes also inhibit carbohydrate metabolic enzyme phosphodiesterase I [162].

Moreover, *Zucker* (fa/fa) rats were administered sodium orthovanadate for 4 months through drinking water. Treatment of vanadate reduce the plasma levels of insulin, triacylglycerols and total cholesterol by 56-77%. Insulin receptors numbers increase 119 % compared to levels in untreated obese animals. The elevated activities of acetyl-CoA carboxylase and ATP-citrate lyase were significantly reduced by vanadate. The messenger RNA for ATP-citrate lyase also decreased in vanadate-treated obese rats back to the lean control levels [74]. Furthermore, due to its peripheral insulin-enhancing effects which lowers insulin demand, vanadium is associated with improved and in some cases, normalized insulin secretion from isolated pancreas [75], and in the increase of insulin receptors numbers in *Zucker* (fa/fa) rats [74]. However, vanadium therapy fails in other metabolic complications of STZ-rats that are corrected by the action of insulin, as the daily weight-gain, that can be only partially corrected by therapy with this trace mineral [76], and the increased rate in muscle protein degradation, where vanadium is ineffective [77]. In addition, it is implicated in the inhibition of protein synthesis, amino acid uptake and mitogenesis, but these effects do not necessarily represent a risk, since the inhibition of growth effects of insulin could alleviate macrovascular disease [78], besides, it has been shown to have anti-tumor properties and is being studied as a potential therapeutic agent in this regard [79-83].

Phosphatases: Reversible protein dephosphorylation is the main intracellular answer for controlling glucose uptake in eukaryotic cells after insulin coupling to its membrane receptor, (Fig. **1**). Diabetic states may arise when signal transduction in a cell breaks down, thereby removing the tight control that typically exists over cellular functions. Vanadium salts and its organic compounds have insulin-mimetic actions related, due at least in part, to their ability as PTPs inhibitors, to improving glucose uptake and lipid metabolism, (Fig. **2**).

However, other actions of insulin as protein synthesis, aminoacid uptake and mitogenesis are inhibited, too. Although not clearly understood, various PTPs are involved and the non-specificity of either inorganic or organic vanadium compounds for PTPs may result in positive as well negative effects on insulin action [78, 84, 85].

Other drugable effects are explained by the fact that the PTPs are signaling enzymes. They are involved in the regulation of numerous cell functions including growth, mitogenesis, motility, cell–cell interactions, metabolism, gene transcription and the immune response [86]. Yet, the predominant effect of vanadium on PTPs and the ability of general tyrosine kinase inhibitors to block the insulin-like actions of vanadium [87-89] indicate strongly that PTPs inhibition is the primary mechanism. Support comes from the irreversible inhibition of PTPs by pervanadate, which is consistent with the fact that it is both, a more potent insulin-mimetic agent and a more powerful PTP inhibitor at the same time [64]. The increased intracellular amount in insulin-resistant adipocytes explains the paradoxical enhanced sensitivity to vanadate [90].

As a specific mechanism of action, blocking PTP-1B has been implicated in normalizing blood glucose levels: PTP-1B is the primary protein tyrosine phosphatase capable of dephosphorylating c-Src in several human breast cancer cell lines and suggests a regulatory role for PTP-1B in the control of c-Src kinase activity [91]. T cell protein tyrosine phosphatase (TC-PTP) plays a significant role in both hematopoiesis and immune function [92], and other PTPs are implicated in the control of cytoskeletal organization and in the generation of integrin-dependent signaling responses in fibroblast [93].

Kinases: Chronic BMOV treatment normalized the insulin stimulated activity of extra-cellular signal-regulated kinase-2 (ERK-2), without having any effect on ERK-1. In contrast to the mitogen-activated protein kinase (MAPK), the activity of $p90^{rsk}$ in response to insulin was low in STZ-diabetic rats and BMOV normalized the kinase activity [94]. Significant stimulation of MAPK and S6 kinase was also observed in cells incubated with vanadium and selenium, which may contribute to the overall insulin-mimetic effects of these elements, but the magnitude and kinetics were different from those of insulin, suggesting a distinct mechanism of action [95]. In our view, insulinomimetics are insulin-like rather than insulin-identical (homologous agonists).

Transcription factors: they are able to recognize and bind to specific sequences of DNA. This specificity is important in regulating the expression of target genes. Regulation and activation of transcription factor is an important element in mediating cellular responses to stimuli. These transcription factors include NF-κB, AP-1 and p53 [29]. Intracellular ROS have also been shown to play a role in certain transcriptional activations by metals [30, 96]. Particularly, vanadate and BMOV induced ROS, reducing the transepithelial electric resistance, causing morphological changes in microvilli and perturbations of F-actin structure [23].

Apoptosis: programmed cell death is a controlled response by which cells can die and yet have a minimal impact on the cells around them. Apoptosis serves the useful purpose of safely eliminating genetically damage cells or cells with developmental errors from the tissues involved. Metals including As, Cd, Cr, Ni, and V have been found to induce apoptosis [31]. Protein kinase B (PKB, also known as Akt) and its upstream signal transducer, phosphatidylinositol-3-OH kinase (PI3K) play an essential role in control of transcription and translation, they both are activated by vanadate [33, 34]. Transcription factor E2F1 is a component of the downstream signals, which are regulated by Akt. The release of E2F1 results in a transition from G_1 to S phase. Vanadate treatment increases the percentage of cells at S phase and triggers phosphorylation of pRb as well as the release of E2F1.

PHARMACOPHORE

A common stable form in aqueous solution is pentavalent orthovanadate, $H_2VO_4^-$, which can trigger insulin-like effects in cells as a competitive, non-selective PTP inhibitor [7, 23, 97]. Vanadate inhibits most strongly those enzymes that form an enzyme-phosphate intermediate. The intracellular inhibition is decreased because vanadate is readily reduced by glutathione and other intracellular reductants [14]. The resulting vanadyl ion is a much weaker inhibitor and also stimulates further metabolic processes

[98], e.g. the cyclic AMP-dependent protein kinase [99]. Consistently, while the pentavalent form (vanadate) predominates in extracellular body fluids, the tetravalent form (vanadyl) is the most common intracellular form.

Oral administration of inorganic vanadium (IV, V) salts (e.g. vanadyl sulfate $VOSO_4$, sodium orthovanadate Na_3VO_4, pervanadate $VO(O_2)_n$ and organic oxovanadium(IV) complexes dotted with binders (e.g. dimetformin, or dicysteine methyl ester, dipyrrolidine N-carbodithiolate, dipicolinate, diacetylacetonate, dimaltoate and alkyl derivatives of the latter two [3]) have shown anti-diabetic activity *in vitro* [8,100-104], *in vivo* [105-115] and even in patients [19,116,117]. Organic vanadium(V) is ineffective except for dipicolinatooxovanadium(V) [3].

Additively, it is a potent, reversible inhibitor of ATPases such as the sodium pump protein (Na^+ + K^+) ATPase [118] of phosphatases [119] and kinases [120]. Another possibility for systemic absorption is transdermal administration: pentavalent bis-peroxovanadium-1,10 phenanthroline, bpV(phen), effectively lowers blood glucose levels in rats. Iontophoresis can significantly reduce the lag time of this response *in vivo* when compared with passive permeation [121]. It is reduced to tetravalent V-species oxidizing CYS215 at the phosphate binding site and described as an irreversible PTP inhibitor [122]. Several patents have been deposed [123-127] and clinical trial status reached with an ethyl derivative of tetravalent organic BMOV [3]. Nowadays, research work on insulinomimetics is branched and V-free compounds have been developed [128-130]. The underlying question is, why not rely on this heavy metal ingredient? Eliminating it does not at all mean having doubts on the anti-diabetic effect which is totally accepted [131], and which is not only known from vanadium [132] but also from zinc [132], tungsten [43], molybdenum [43], cobalt [133] and chromium [134]. Ethnopharmacological studies reveal that V and Cr containing plants have been used for diabetes treatment for a long time, e.g. *Eugenia jambolana* seeds or *Atriplex halimus L*. [135].

Speaking of chronology, in 1985, the scientific group among McNeill, BC, Canada, claimed first reports on *in vivo* blood glucose lowering and insulin saving after oral administration of inorganic salts (vanadyl, vanadate) as insulinomimetics [136]. Then they reported on patented BMOV for "increased potency of vanadium using organic ligands"[137]. It is worthwhile to analyze this potency as non-selective PTP inhibitor: it does not refer to pharmacodynamics but rather to pharmacokinetic improvement (better bioavailability) upon oral administration. In 2000 and 2003 findings confirm, passive membrane diffusion eased by anion channels was accounted for the higher uptake of more lipophilic aromatic substances like VO_2dipic$^-$ [3] and BMOV *vs.* inorganic salts [8].

The discussion on chelate stability strongly suggest, that if citrate is met, it will certainly react for example with BMOV, understanding that it is transported in the blood serum, presumably in the form of its ternary species with citrate. Inorganic vanadyl(IV) sulfate as well as organic BMOV(IV) are oxidized to state (V), probably by absorbed atmospheric oxygen in the aqueous solution [3, 8]. In erythrocytes, vanadate(V) is apparently quickly reduced into oxovanadium(IV) by glutathione, a key player in vanadium metabolism which itself can act as a ligand for the generated VO^{2+} cation [14]. Missing data for organic vanadium complexes let us recommend reduction experiments for organic vanadium(V).

Our tables show the use of organic ligands seems to decrease the toxic effects, but only assuming that the observed effects are due to the administered complexes. However, in the light of the chelate and redox versatility of vanadium, we examine the prodrug hypothesis that inappropriate (even inactive precursors) but unstable complexes would undergo re-chelatization and/or oxidation to form the active moiety. Then, less toxic effects would imply less anti-diabetic effects, too, because of more stable organic precursors. Indeed, Crans [3] already looked for a correlation between stability and effectiveness. But Crans seems to overlook in her ranking lists of four-membered series, that it mixes up *in vitro* and *in vivo* data (last line in Table **3** [3]). The key to understand a dose – response mismatch is already mentioned above: given oral administration (which is not explicitly stated), liberation and absorption fluctuate, different bioavailability hampers correlation, just like in any bioequivalence study (*in vivo* versus *in vitro* dissolution tests) for generic drugs. We suggest to compare the magnitudes of hydrolytic products and vanadyl VO^{2+} with efficiency of i.v. administered equimolar. Correlating Crans' Table **3** [3] with Table **2** in reference [7] in conjunction with the correct complex stabilities for VO(edta) $3.80x10^5$ < $VO(maltol)_2$ $1.05x10^7$ < $VO(citrate)_2$ $3.16x10^{11}$ [9] we conclude: "the less stable the organovanadium complex, the more effective". Therefore, any organovanadium complex should be regarded as a non-active precursor (prodrug). In 2003, a trigonal-bipyramidal vanadium oxide was detected to bind *in vitro* to the active site of PTP1-B and not administered BMOV [97].

SPECIFICITY AND SELECTIVITY

Dissociation of toxic side effects and desired blood glucose lowering effect is the major concern for the future drug development for organovanadium compounds to become an attractive option, i.e. drugable. The well-conserved phosphate binding cleft within the PTP family initially led to the belief that specificity for phosphate imitating inhibitors would be difficult to achieve. Experimental structure elucidation of V-free substrate-analogous bound to PTP-1B reveals that there are regions within the binding cleft that the phosphate does not occupy, (Fig. **4a**).

Regio-selectivity is possible since the unoccupied hydrophobic pockets show structural diversity between members of the PTP family (F181 with T46 or V49, Q262; A272, I219, Q262; 1ptu numbering). Amino acids 214 to 222 form the PO_4-binding loop, the so-called PTPase signature motif, VAL or HIS214, CYS215, X216, X217, X218, X219, X220, ARG221, THR or SER222, X being not a conserved amino acid, taking the numbering scheme from 1PTP-1B (PDB entry 1ptu) [28].

It has been shown that ligand binding by non-vanadium organic phospho tyrosine analogs is stronger, achieving ten times lower concentrations $0.2x10^{-6}$ M than vanadate with $1\text{-}2x10^{-6}$ M [138].

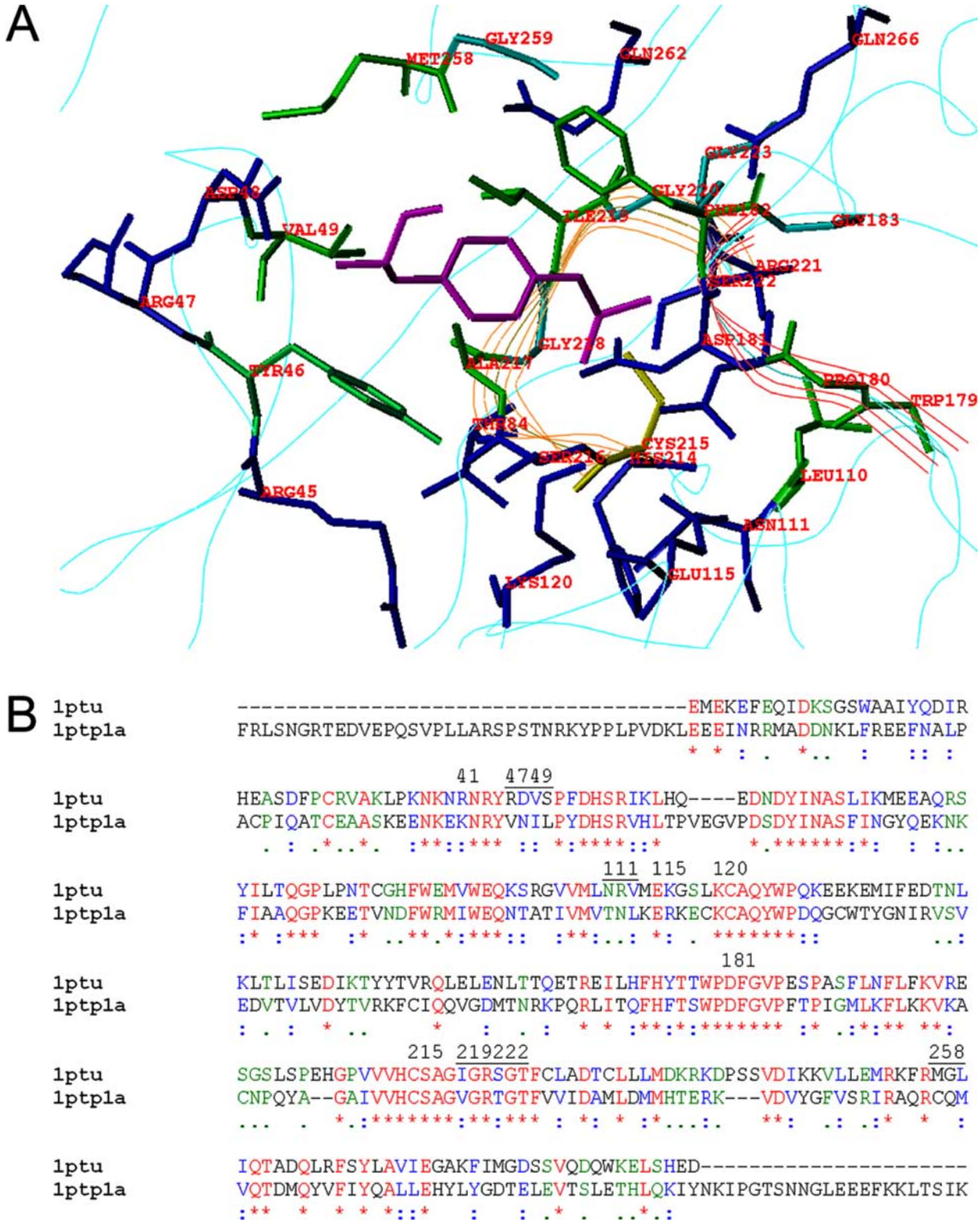

Fig. (4). Visualization of regiospecifity by computer model [28, 159] and multiple sequence alignment [160]. Above: hydrophilic (blue) and lipophilic (green) amino acids around the phosphate binding loop (orange ribbons), WPD loop (red ribbons) with phospho-tyrosine PY (magenta). Hydrogen atoms omitted. Below: underlined id numbers show not conserved amino acids in hydrophobic pockets (V49, A217, I219, M258) and polar regions (R41, R47, D48, N111, S222) of PTP1B (1ptu numbering).

DRUG DESIGN

To our experience, the major challenge in vanadium – phosphatases modeling is twofold: firstly, to dispose of a valid parameterization for V in force fields or semi-empiric models; second, to come across with PTP's conformational flexibility (WPD-loop *vs.* PTPase signature motif), facing the necessity to make spatial distinctions between the family

members under crystallographic precision [138,139]. As illustration: the WPD loop closes after binding in 1bzc [140], 1bzj [140] and 1ptu [28] with a short heptapeptide ligand (pTyr), (Fig. **4**). In presence of other ligands, some do not contain pTyr, such as 1bzh with a cyclic peptide [140], and 2hnq with WO_4^- anion as a ligand [141], the WPD loop remains in opened conformation. Yet, the most valuable information about the binding mechanism was achieved by molecular dynamics studies [142-144]. The interaction site diversity (multiple sequence alignment analysis) provides opportunities for the discovery or design of selective small molecule competitive inhibitors, (Fig. **4b**).

CONCLUSION

PTPs represent a novel platform for drug discovery [145, 146]. Taken together the information on PTP-inhibition, the molecular mechanism of action for organovanadium complexes seems to be that of a prodrug: under an adequate complex life (for liberation, gastrointestinal absorption and distribution), they show stronger potency than less lipophilic inorganic V-ions because of their increasing cell permeation. Thereafter, they all become instable by intercellular redox reactions and ligand replacements, and end up as vanadium oxide coordinated with amino acids and water molecules in order to reversibly and unselectively block the phosphate binding site of PTP-1B. Phospho-mimetic effect is our more suitable expression than the word insulin-mimetic, because of variable oxidation and coordination states characterizing complex aqueous chemistry.

The pros and cons for developing vanadium compounds as anti-diabetic drugs are: (i) suggested therapeutic range is slightly higher (tenfold) than for recommended long term tolerance; acute toxicity is less a problem: with caution (crossing species data!), human LD lies two order of magnitude higher than doses reported in clinical trials so far. Beware, dose-independent (immuno- gonado-) toxicity not evaluated here. (ii) certain V-free pTyr analogs are as potent as vanadium or even stronger. (iii) vanadiumorganic compounds have to be developed to use unoccupied pockets *versus* certain V-free pTyr analogs are already regio-selective. (iv) Theoretically, it is conceivable to design such a molecule stable enough to enter the cells, dock to PTP-1B active site selectively with or (!) without changing oxidation and coordination to interact with the phosphate binding loop and WPD loop and two varying hydrophobic pockets. As yet, vanadium compounds have to be considered as prodrugs delivering vanadium oxide bound into the active site pocket, unless a safe, stable and specific organic chelator is developed to prove the contrary. (v) Yet, vanadium has a broader spectrum imitating phosphates in signal transduction, so it is wise to look for new targets like (de-)phosphorylation-(dis-)regulated cancer where toxicity concerns show lower clinical priorities etc.

The vanadium drug research should go on for its potential targeting phosphate signal transduction, which is in need of molecular differentiation to better understand cellular physiopathology. At least, vanadium compounds can be used as pharmacological *in vitro* test substances in phosphatase – kinase research or with an altered indication in terminal anti-neoplastic drug therapy where long term side effects are of no concern.

ABBREVIATIONS

Acac	=	Acetylacetonate (-1)
acetyl-CoA	=	Acetyl coenzyme A
ADP	=	Adenosine diphosphate
Akt	=	A serine/threonine kinase (58 kD) with SH2 and PH domains is involved in stimulation of Ras and control of cell survival
AP-1	=	A Transcription factor, formed from a heterodimer of the products of the proto-oncogenes fos > fos and jun > jun.
BMOV	=	Bis-maltolateoxovanadate, $VO(maltolate)_2$
bw	=	Body weight
DM I, DM II	=	Diabetes Mellitus type 1 (insulin dependent), type 2 (not -)
EGP	=	Endogenous glucose production
ERK	=	Extra-cellular signal-regulated kinase
G-6-Pase	=	Glucose-6-phosphatase
GDP	=	Guanosine diphosphate
IDDM	=	Insulin dependent DM
LD50	=	Letal dose causing the death of 50 % of all animals exposed
MAPK	=	Mitogen-activated protein kinase
NADH	=	Nicotinamide adenine dinucleotide (reduced form)
NF-κB	=	Redox-sensitive pleiotropic transcription factor
NIDDM	=	Non-insulin dependent DM
p53	=	A 393 residue (in humans) phosphoprotein that is a common tumour antigen, expressed in many transformed cells
peroxovanadium	=	See Vanadium
pervanadate	=	See Vanadium
PDB	=	Protein Data Base
PEPCK	=	Phosphoenolpyruvate carboxykinase
PI3K	=	Phosphoinositol-3-OH kinase
PKB/Akt	=	Protein kinase B
PTP	=	Protein tyrosine phosphatase
PTP1B	=	Protein tyrosine phosphatase 1B
PTPs	=	Protein tyrosine phosphatase family enzymes

pTYR	=	Phosphate carrying tyrosine, abbreviated: pY
ROS	=	Reactive oxygen species
STZ-diabetic	=	Diabetes induced by streptozotocine
T1DM	=	Type 1 Diabetes Mellitus, same as IDDM
T2DM	=	Type 2 Diabetes Mellitus, same as NIDDM
TC-PTP	=	T cell protein tyrosine phosphatase
Tiron	=	4,5-Dihydroxy-1,3-benzenedisulfonic acid, disodium salt
V	=	Vanadium, in honor of the beautiful aspects of its various crystals the name was according to the Nordic god of beauty Freya Vanadis by the Swedish chemist Selfström in 1830.
Vanadate	=	Anion with oxidation state +5 (V), $H_2VO_4^-$
Vanadyl	=	Cation with oxidation state +4 (IV), $VO(H_2O)_5^{2+}$, abbreviated VO^{2+}, oxovanadium(IV) or just vanadyl
VO_2dipic$^-$	=	Dipicolinateoxovanadium (IV)
$VO(acac)_2$	=	Diacetylacetonateoxovanadium (IV)
$VO(maltolate)_2$	=	BMOV, bis-maltolateoxovanadate
Peroxovanadium	=	Various anions (V), like VO(O2)n, abbreviated pervanadate
WPD loop	=	TRP179-PRO180-ASP181 segment in PTP1B (1ptu numbering)

ACKNOWLEDGEMENTS

H.P.T. Ammon, N. Lembert, University Tuebingen, and R. Lammers, Med. Clinic Tuebingen, Germany, for discussion; C. and F. Scior, Columbus, OH, for language revision.

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