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Review article

Vanadium in diabetes: 100 years from Phase 0 to Phase I

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Abstract

A little over one hundred years ago, a vanadium-containing compound was assessed clinically for use in treatment of human diabetic patients. The results were somewhat ambiguous, but nonetheless, intriguing. In 2000, the first Phase I clinical trial of a designed vanadium-based pharmaceutical agent (bis(ethylmaltolato)oxovanadium(IV), BEOV), was completed by Medeval Ltd., Manchester, UK. Results here, too, were promising, but not without some difficult remaining questions. In this review, we look back at the many questions asked and answered regarding vanadium's glucose-enhancing potential, its biodistribution and biomolecular transformation, and its mechanism(s) of action, and consider some of the newest developments in the field, including novel delivery methods for vanadium in diabetes treatment.

Keywords: Vanadium; Bis(ethylmaltolato)oxovanadium(IV); Diabetes mellitus; Biodistribution

1. Introduction

The first report of vanadium salts being used as a metallotherapeutic appeared in 1899 [1,2]. Consistent with medical trials of that era, Lyonnet and his colleagues first tried the proposed drug on themselves, then on 60 of their patients (three of whom were diabetic) over a period of some months. They described what might be considered today a ''Phase 0'' clinical trial in somewhat vague terms: 4–5 mg sodium metavanadate (before meals) every 24 h, three times per week, with resulting two out of the three diabetic patients said to have obtained a slight, transient, lowering of sugar levels. No ill effects were noted in any of their patients.

One hundred years later, the first Phase I clinical trial of a designed vanadium complex was completed (first reported at ICBIC-11, July, 2003 [3] and summarized in [4]. Safety, tolerability, pharmacokinetics and bioavailability of escalating doses of a vanadium complex, bis(ethylmaltolato)oxovanadium(IV) (BEOV) (Fig. 1), intended for therapeutic use in diabetes mellitus, were assessed in a total of 40 non-diabetic subjects. The highest dose was 90 mg BEOV, administered orally as a single dose, and there were no trial-associated adverse effects.

Now, six years later, we seem to be no closer to bringing a vanadium-based hypoglycemic agent to market as a prescription drug; yet so-called 'organo-vanadium compounds' are appearing with increasing regularity as part of many over-the-counter vitamin and mineral supplements [5–7], also apparently with no ill effects. But will vanadium compounds, that are orally available, ever replace insulin (that has to be administered by injection) for millions of diabetic patients?

Our intention in this review article is to focus on the questions asked and answered (mostly over the last 25 years) about the design of vanadium compounds for diabetic treatment, and highlight some of the ongoing areas of contention surrounding this endeavor. A spate of recent reviews will ensure that the interested reader can delve deeper into selected aspects of vanadium's bioinorganic chemistry, e.g., biological and medicinal aspects of vanadium

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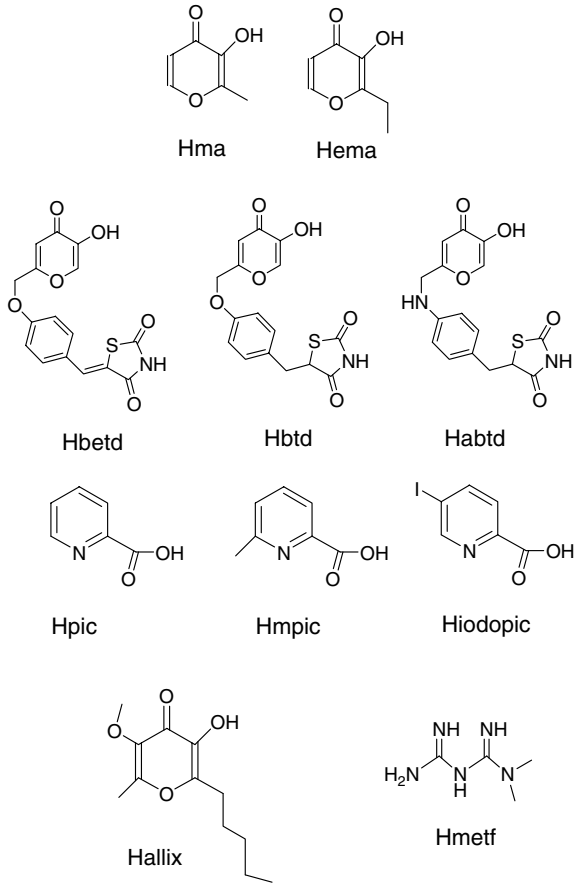

Fig. 1. Structures of representative ligands discussed in this review. All shown here are monoprotic and have been used as monoanionic coordinating ligands in vanadyl complexes of the type, VOL_2, designed as vanadium-based therapeutic agents for the treatment of type 2 diabetes mellitus. Hma = maltol; Hema = ethylmaltol; Hbetd = ((±)-5-[4-(5-hydroxy-4-oxo-4H-pyran-2-ylmethoxy)benzylidene]thiazolidine-2,4-dione; Hbtd = ((±)-5-[4-(5-hydroxy-4-oxo-4H-pyran-2-ylmethoxy)benzyl]thiazolidine-2,4-dione; Habtd = ((±)-5-{4-[(5-hydroxy-4-oxo-4H-pyran-2-ylmethyl)amino]benzyl} thiazolidine-2,4-dione; Hpic = picolinic acid; Hmpic = 6-methylpicolinic acid; Hiodopic = iodopicolinic acid; Hallix = allixin; Hmetf = metformin.

chemistry [8]; vanadium compounds in the treatment of diabetes [9,10]; comparative pharmacokinetics of several vanadium compounds [11]; biochemistry of vanadium compounds, with emphasis on the 'phosphate analogy' [12]; effects of vanadium on the insulin signaling cascade [13]; and coordination chemistry and metabolic pathways of vanadium compounds for diabetes treatment [14].

2. The first sixty years

The discovery of insulin in 1922 [15], and its almost immediate adoption as the treatment of choice in diabetes mellitus, led to a long hiatus for medicinal use of vanadium compounds. Early interest in vanadium as a metallotherapeutic waned, even as exploration of vanadium's biological effects in plants and animals continued. For example, during the first half of the 20th century, Bertrand, *père et fils*, published 18 articles on perceived biological activities of vanadium (as reported in [16]), particularly in plants and fungi. Increased oxidation of phospholipids was observed in tissue cultures upon addition of sodium metavanadate or vanadium acetate [17] in millimolar concentrations. This observation, along with other reports of vanadium's ubiquitous nature and pharmacological effects [18–20], led to a series of clinical trials in humans in the mid-century, not for diabetes treatment, but as potential cholesterol-lowering pharmaceutical agents [21–23].

Vanadium salts proved to have only a very modest (though statistically significant) inhibitory effect on serum cholesterol; however, it is interesting, from our current vantage point, to note the absence of any negative effects, even at quite high doses of "di-ammonium vanado-tartrate": 125 mg/d for 5 months in 10 patients, in one study [22]; the same for 6 weeks in five medical students in another [21]; and a total administered dose of 8375 g over a 94-day experimental period in a third [23].

During roughly this same period (early- to mid-1960s), there was also a growing appreciation for the complexity and versatility of oxovanadium(IV) and (V) coordination complexes. Vanadyl bis(acetylacetonate) was synthesized and characterized [24]; a tetrahedral vanadate, an oxyanion of vanadium(V), was first described [25]; spectral characterization of vanadium(IV) and (V) complexes became possible [26–29]; and the characteristic eight-line vanadyl EPR spectrum was described [30]. The coordination chemistry of oxovanadium(IV) was first summarized in 1965 [31]. Vanadium's strikingly complex solution chemistry was reviewed a few years later [32].

3. Mid-1960s to mid-1980s – from "abnormal trace element" to "versatile biochemical effector"

The stated purpose of a review article entitled "Abnormal Trace Elements in Man – Vanadium" was to consider whether vanadium might be an essential element in humans [16]. At the time that this monograph was published, measurement of vanadium in biological samples was still exceedingly difficult, as emission spectrographic analyses were neither very precise nor necessarily accurate at the nanomolar level and below. Nonetheless, Schroeder and co-workers were able to conclude that trace amounts of vanadium were ubiquitous, apparently under homeostatic control, and had a 'low order of toxicity', all of which tended to support (but not prove) its essentiality [16]. Hence, further studies were undertaken to elaborate vanadium's metabolism [33,34], effects on growth [35], and possible deficiency symptoms [34].

3.1. Vanadium and glucose metabolism – a serendipitous discovery

The discovery that vanadium(V), as vanadate, was an extremely potent enzymatic inhibitor was completely unanticipated. A number of laboratories world-wide had

noticed that a particular commercial preparation of ATP (from Sigma Chemical Co.) gave anomalously low catalytic rates in a standard ATPase assay [36–38]. As described by Josephson and Cantley [38]: "At concentrations between 10^{-8} and 10^{-7} M, [a] muscle derived inhibitory factor (MIF) produces 50% inhibition of the enzyme.... While this paper was in press we were able to determine that MIF is vanadium in the 5+ oxidation state (vanadate). ... It exhibits a K_I of 40 nM for the dog kidney (Na–K)ATPase under standard assay conditions and its inhibition is reversed by norepinephrine" [38].

Subsequent studies determined that this inhibition of cation transport originated from the cytoplasmic side of the cell membrane [39,40], and that the inhibition involved vanadate preventing a de-phosphorylated enzyme conformational change [41]. Similarity in size and charge of vanadate and phosphate, with vanadate just enough larger to irreversibly inhibit phosphate-dependent enzymes, resulted in widespread use of vanadates as protein tyrosine phosphatase inhibitors for all manner of cell assays (as reviewed in [42]). Reduction of vanadate to vanadyl in the cytoplasm, accompanied by intracellular protein binding, relieved inhibition of the ATPase [43].

Other research highlighting a range of physiological and pharmacological effects of vanadium (reviewed in [44–46] and the subject of a symposium, "Cardiac Effects of Vanadate", October 26–27, 1979 in Munich) [47], suggested to Edward Tolman and co-workers [48] that vanadium might have profound effects on another energy source [besides cholesterol and ATP], namely glucose. This was indeed the case, as was abundantly demonstrated in a variety of cell types over the ensuing half-dozen years [49–51]. (For review of early studies of vanadium salts as anti-diabetic agents, see [52,53], and of vanadium's more general physiological, metabolic and enzymatic effects, both stimulatory and inhibitory, see [54]).

By the mid-1980s, vanadium compounds were recognized as occupying a unique position among metallopharmaceuticals, both for reasons of their potent pharmacological effects and for their physiological interconversion between cationic (vanadyl) and anionic (vanadate) species [55–61]. Vanadium was indeed an ultratrace metal with "an elusive biological function" [44] and many possible regulatory roles in the body [54], often related to glucose and lipid metabolic pathways.

3.2. Vanadium's insulin-like effects demonstrated in vivo

If so many of vanadium's *in vitro* effects were reminiscent of those of insulin under similar conditions, how would it behave *in vivo*? Animal models of diabetes, including alloxan-induced and streptozotocin(STZ)-induced diabetes in rats, were making it increasingly possible to try out candidate anti-diabetic therapeutics [62]. The pioneering experiment of McNeill and co-workers [63], adding sodium orthovanadate to the drinking water of streptozotocin (STZ)-diabetic rats for several weeks, was breathtaking in its simplicity, and even more so in the clarity of the results: a dilute solution of a vanadium(V) salt taken orally alleviated many of the signs and symptoms of STZ-diabetes with few negative side effects. This was really an unanticipated discovery, as the *in vitro* insulin-like effects noted in the late 1970s and early 1980s were observed at vanadium ion concentrations in the 0.2–3 mM range. On the other hand, *in vivo* tissue concentrations of vanadium following short-term supplementation trials were shown to be in the nanomolar to micromolar range [64–66]. Nonetheless, the initial experimental results in diabetic rats [63] were corroborated independently by diverse investigators internationally, e.g., in Poland [67], Israel [68], Belgium [69], Spain [70], USA [71], and Japan [72] (reviewed in [73] and [74]).

An apparent aversion to solubilized vanadate was resolved by substituting vanadyl sulfate for sodium orthovanadate, with fewer negative side effects, and a lower mortality from hypoglycemia and gastrointestinal distress [75–77]. Vanadium salts, at doses ranging from 0.1–0.7 mM kg^{-1} d^{-1} [78–82], in a variety of animal models of diabetes [79,83], normalized blood glucose and lipid levels, corrected thyroid hormone deficiency, improved insulin sensitivity, and prevented or reversed secondary complications, such as cardiomyopathy, cataract development, impaired antioxidant status and excessive food and fluid intake [84,85], reviewed in [86].

3.3. Dose dependence? Dichotomy of response

There was no doubt that vanadium ions could have powerful pharmacological effects *in vivo*. But how could vanadium be both a potent inhibitor of sodium pump enzymes (Na^+,K^+ ATPase and Ca^{2+}ATPase), while at apparently similar doses, alleviating most of the symptoms of diabetes mellitus? Addressing the question from a toxicological point of view [87] led to the inevitable conclusion that rats treated with vanadyl sulfate at greater than 2 mM V kg^{-1} body weight (and even lower doses of vanadate salts) should all die of vanadium toxicity (based on toxicity tables from [88]). Two-week LD_{50} values for i.p. administration were even lower than those for oral gavage administration, with an i.p. LD_{50} of 0.15 mM kg^{-1} body weight for sodium metavadate in an acute toxicity trial [87].

The answer to this conundrum lay in a re-examination of the evidence from a metabolic and pharmacokinetic point of view [89–92]. From these studies, it became clear that vanadium compounds given orally over an extended period of time (e.g., "in the drinking water" [63]) are absorbed at a very low level [93], rapidly associate with serum proteins [91,94,95], change oxidation state in the body [58–60], and are cleared moderately quickly [33,92]. The schematic outline offered by Chasteen et al. in 1985 [89] remains essentially unchanged today (see Fig. 2 for current rendition) [14]. Pharmacokinetic analyses predicted that most of an inorganic vanadium dose would be excreted rapidly, having never been absorbed at all

[96–98]. Thus, an apparent overlap between toxic and therapeutic doses disappeared.

4. Modification of vanadium's potency by ligand complexation

The question then became, "if vanadium ions are effective, but too close for comfort to levels at which adverse effects are observed, is there some way to chemically improve potency of these putative anti-diabetic agents?" This question was answered with a resounding, "Yes!" with the report of bis(maltolato)oxovanadium(IV), BMOV, in 1992 [99] (Fig. 2). BMOV was two to three times more effective acutely than vanadyl sulfate as a glucose-lowering agent, was better tolerated than inorganic vanadium salts, and resulted in reliable glucose-lowering in all animal models of diabetes in which it was tested [100,101], reviewed in [86] and [102]. BMOV could be administered in drinking water; thus avoiding a requirement for oral gavage administration of a suspension, as was the case with a vanadyl-cysteine methyl ester, $VO(IV)(Cys\text{-}Me)_2$ that had shown promise a couple of years prior, with efficacy equivalent to vanadyl sulfate [103]. A pharmacokinetic study performed with carrier-added ^{48}V-BMOV and $^{48}VOSO_4$ [104] conclusively demonstrated improved tissue uptake of vanadium from the vanadyl ion complexed to the hydroxypyrone, maltol, an approved food additive, compared to its inorganic congener [105].

Improved potency and efficacy by judicious ligand binding of vanadyl ions have been achieved by numerous other investigators [106–109] and this remains an area of active investigation today (reviewed in [10]). Other oxidation states of vanadium – vanadium(III) and vanadium(V) – have also shown promise as potential hypoglycemic agents (e.g. [110] for V(III) and [111] for V(V); however, to date, none has surpassed BMOV in terms of *in vivo* efficacy in chronic trials (reviewed in [14]).

Initially hailed as a quantum leap forward [in terms of potency], the discovery and exploration of peroxovanadium complexes (also known as pervandates or peroxovanadates) [112–115]), has proven to be more interesting as a tool to probe vanadium's mechanism of action than as a new class of diabetes therapeutic agents, due to lack of oral bioavailability [116].

A key question, of course, with respect to continuing development of vanadium compounds as therapeutic agents for diabetes treatment, is "how will these work in humans?" The first trials of inorganic vanadium compounds in diabetic patients were completed in the mid-1990s [117–120] at the Joslin Diabetes Center in Boston, MA and at the Diabetes Research Center, Albert Einstein College of Medicine, NY. Adverse effects were confined to minor gastrointestinal distress, and doses up to 100 mg vanadium per day (2 mmol V d^{-1}, as either vanadyl sulfate or ammonium metavanadate, in separate trials) were well-tolerated for up to four weeks [121] and did not increase oxidative stress in individuals [117]. A longer study (6 weeks' duration) at a gradually introduced dose of ~1 mmol V d^{-1}, as vanadyl sulfate, was well-tolerated by 10 of 11 type 2 diabetic patients [122]. In all cases, there was, at best, modest improvement in insulin sensitivity and glycemic control at these doses [119], and the inter-individual variability in response was disturbingly high [119,121,122].

The question, "if vanadium ions are effective in alleviating diabetic symptoms, why are the results so variable?," had arisen previously on repeated occasions in animal

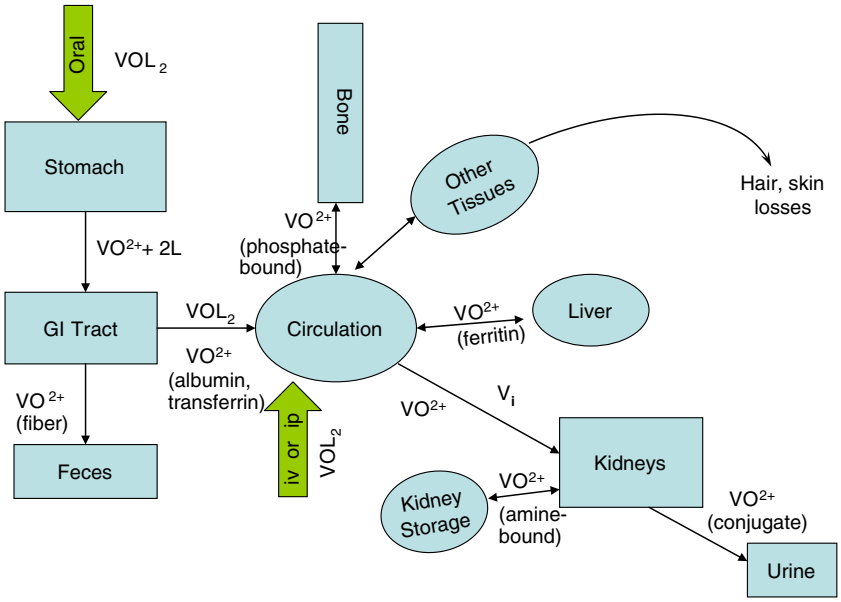

Fig. 2. Schematic model of absorption, distribution, metabolism and excretion of vanadium compounds of the general formula, VOL_2 [14]. Reprinted with permission from [14]. Copyright (2005) American Chemical Society.

trials. In some cases, experimental animal groups were divided into 'responders' and 'non-responders' [101,123], as there seemed to be a distinct dichotomy of response: either an animal responded to vanadium treatment early on, or it might never respond at all [82]. Vanadium's pharmacological effects in animals varied with mode of administration, with profound differences in efficacy among an i.p. dose, an oral gavage dose, and a dose taken in slowly over the course of feeding (*vide supra*) [103,124]. Other delimiters were found to be the initial oxidation state of the vanadium compound [96,125], how severely diabetic the animals were at the start of treatment [82,126], the presence or absence of endogenous insulin stores [127,128], how much sucrose was included in the diet of experimental animals [129] and renal insufficiency, which could increase serum and tissue vanadium independent of other factors [130]. Aside from physiological factors, biomolecular processes also play a role in determining the fate of exogenous vanadium compounds [43,61,131] (*vide infra*).

5. Absorption, distribution, metabolism and excretion (ADME) of vanadium complexes in chronic treatment

5.1. BMOV/BEOV pharmacokinetics

The proposed use of vanadium compounds as chronic treatment alternatives for diabetes [132–138], with anticipated administration over many years, necessitated an improved understanding of these compounds' absorption, distribution and excretion patterns. BMOV, and its ethylmaltol analog, bis(ethylmaltolato)oxovanadium(IV), BEOV, (a.k.a. KP102) were at the forefront of these ADME studies.

The carrier-added, direct comparison study between ^{48}V-BMOV and $^{48}VOSO_4$ [104] demonstrated a similar pattern of biodistribution to that of inorganic vanadium salts observed earlier [33,97,98,139–141], especially with regard to the order of relative accumulation, with bone > kidney > liver. A long half-life of elimination (>10 d for ^{48}V in bone after a one-time 10 μM dose by oral gavage in rats) and low level of absorption (with bone retaining ~0.1% of an oral dose/g tissue 24 h after an oral dose of $VOSO_4$), were corroborated for $VOSO_4$ in the carrier added study [104], and shown to be enhanced by BMOV, which had a two-fold longer half-life of elimination from bone. A clear advantage of BMOV over $VOSO_4$ was that uptake of ^{48}V was two to three times greater in most tissues 24 h after an oral dose equivalent to approximately 10 μmol vanadium.

What the carrier-added ^{48}V study could not show was whether the hydroxypyrone complex of vanadyl stayed together after oral or i.p. administration. Radiolabelled BEOV studies showed that most (if not all) labelled BEOV is decomposed upon entering the bloodstream [109], with intact BEOV persisting for less than an hour after an oral gavage treatment in rats. Blood-circulation monitoring studies, pioneered by Sakurai and co-workers [142,143], in which the fate of the vanadyl ion is tracked by EPR, also indicated rapid disappearance of vanadyl from the bloodstream, especially when vanadyl is introduced as a solution of vanadyl sulfate, with an i.v. dose having a vanadyl half-life of just over 5 min [142].

5.2. Biomolecular transformation/solution chemistry/ligand substitution

The interaction of pro-drugs with serum proteins and small molecular entities, e.g., citrate and ascorbate ions, is an important aspect of drug metabolism, capable of strongly affecting the distribution, biotransformation and ultimately the mechanism of action of pharmaceutical agents [144], and vanadium compounds are no exception [145,146]. Speciation modeling studies of BMOV, vanadyl picolinate, and vanadyl 6-methylpicolinate [146] concluded that 90% of vanadyl ions in circulation would be bound to citrate anions at a compound concentration of 10 μM, with a metal ion to ligand ratio of 1:2:2. Strong complex formation with high molecular weight serum proteins, transferrin and albumin, was also predicted. Further studies, using stopped flow spectrophotometric kinetic characterization of complex formation for BMOV [147], concluded that maltol would rapidly be replaced by stronger binding ligands. Also, pH-potentiometric and EPR spectroscopic studies confirmed the likelihood of both ternary complex formation and predominant transferrin binding, with oxalate, lactate, citrate and phosphate ions being the predominant low molecular mass binders [148].

Interactions of BMOV with the serum proteins, apotransferrin (apoTf) and albumin (HSA), were studied in more detail using variable temperature EPR [149]. Transferrin concentration is ~37 μM in plasma; however, its metal binding sites are only ~30% occupied by Fe(III), leaving a significant metal binding reservoir of sites (~50 μM) available for reaction with metal-based drugs. BMOV binding to human serum apo-transferrin (apo-Tf) resulted in frozen and room temperature EPR spectra that were indistinguishable from those for vanadyl sulfate ($VOSO_4$) binding to apo-Tf. This binding did not take place in the absence of bicarbonate ion, as required for vanadyl ion binding, analogous to the situation with Fe(III).

By contrast, the interaction of BMOV with human serum albumin (HSA) was indicative of potential adduct binding and produced an EPR spectrum distinctly different from that of $VOSO_4$ and HSA. Adduct formation with HSA could both protect a vanadyl complex against oxidation [150] and increase relative efficacy by slowing transit time through the bloodstream [143]. Kiss et al. [146] predicted that if the stability of VO-HSA binding were increased to ~1/6 that of apo-Tf, the proportion of vanadyl ions bound to HSA would increase from 0% to 80%. Ternary and/or adduct complex formation could serve to increase the HSA-bound fraction, and thus slow systemic circulatory clearance of the vanadyl ion, leading to increased *in vivo* efficacy [151], as proposed also for several

picolinato-vanadyl complexes [142,143,152], and for vanadyl acetylacetonate [153,154].

A question of considerable importance at this point, then is, "if insulin-mimetic complexes dissociate at or before absorption, is there an optimal formulation for a vanadium pro-drug?" A tendency of late to refer to vanadyl complexes as 'organo-vanadium', as if all complexes were basically the same, belies the observed broad spectrum of responses – all the way from none at all, to 100% glucose-lowering – in an array of coordination complexes of vanadyl with carbon-based ligands [10]. As with other medicinal inorganic compounds, the whole compound needs to be taken into account when evaluating pharmaceutical efficacy, as well as toxicity [155].

The complex biochemistry of vanadium ions, with extensive binding to systemic and intracellular ligands, both large (transferrin, albumin) [156–158] and small (glutathione, citrate, ascorbate, lactate) [159–161], has also tended to confound efforts to pin down its preferred biological function [162] (*vide infra*).

Clearly, compounds that do not dissociate readily tend to have no insulin-mimetic effect *in vivo* [10,163,164]. Among complexes with fairly similar short-term potencies, such as BMOV, BEOV, $VO(pic)_2$, $VO(6MPA)_2$, $VO(acac)_2$, and $VO(Etacac)_2$ [152,165,166], the deciding factor may relate more to the ligand itself than to the complex. Use of a non-toxic ligand (such as maltol or ethyl maltol) is thus preferable to use of a ligand with a known problematic toxicity profile, e.g., acetylacetonate [167] or picolinic acid [168].

5.3. Mechanism(s) of action

Inevitably, the question, "how does it work?" has arisen repeatedly with respect to vanadium complexes and anti-diabetic effects. In the case of peroxovanadates, that question has largely been answered: the highly potent, irreversible inhibition of insulin receptor protein tyrosine phosphatases (PTPases) [169] dominates all other biochemical effects (Alan Shaver, personal communication). For vanadyl complexes, the situation may be more complex. One suggestion is that *in situ* formation of peroxovanadates leads to inhibition of PTPases in the insulin signaling cascade (Forrest Nielsen, personal communication). Another is that vanadyl stimulates cytosolic protein kinases, thus bypassing the insulin receptor altogether [170–172]. Other important stimulatory effects are clearly at play as well, namely GLUT4 translocation from intracellular compartment to the plasma membrane [162,173]. Most likely, some combination of effects is involved [174,175] (for a complete recent overview, see [13]).

5.4. Recent advances in design of insulin enhancing vanadium-based therapeutics

Beyond ensuring sufficient stability to avoid hydrolytic degradation prior to absorption [111,176], and use of non-toxic ligands, there are still choices available to potentially enhance the therapeutic effect of a vanadium-based pharmaceutical. Bi-functional vanadium compounds that have been tested as insulin-enhancing agents include vanadyl-metformin and close analogues [177], vanadyl thiazolidinediones [178], vanadyl curcumin [179,180], and bis(allixinato)oxovanadium(IV) (Fig. 1) [181]. The bi-functionality of the first two is based on use of known oral hypoglycemic agents (or close analogues) as ligands, and of the second two on use of known antioxidants. Curcumin is both an antioxidant (reviewed in [182]), and an antidiabetic agent in its own right [183]. It is isolated from turmeric, a spice extracted from the rhizome of *Curcuma longa* L. Allixin is the active principle isolated from garlic (*Allium sativum*) and, like maltol and ethylmaltol, is also a 3-hydroxy-4-pyrone [105].

Ensuring a balanced lipophilicity and hydrophilicity is also important for orally available vanadium-based pharmaceuticals [11,153,184]. In recent variants of picolinato vanadium(V) complexes [176], a series of 5-carboalkoxypicolinato-vanadium(IV,V) complexes were synthesized and underwent preliminary testing as insulin-mimetic complexes [185]. Along with a favorable hydrophilic/lipophilic balance, these complexes were sufficiently stable that dissociation in the stomach would be avoided, thus minimizing gastrointestinal distress, while taking advantage of the more rapid cellular uptake by vanadate, compared to vanadyl, compounds [8,124,186,187]. Once inside the cell, the vanadate complex undergoes ligand substitution and vanadium(V) is reduced to an oxovanadium(IV) species [187].

5.5. Alternative delivery methods – iontophoric; enteric-coating; transdermal

In addition to ligand complexation, several other methods have been tried for increasing vanadium bioavailability and decreasing the incidence of gastrointestinal irritation, usually the principal adverse effect. One was use of hydrophobic carriers, principally monohydroxamates, administered side-by-side with vanadyl sulphate [106,188,189]. Another method [not initially identified as such] was co-administration of Tiron, (4,5-dihydroxy-1,3-benzenedisulfonic acid), a water-soluble, disulfonated catechol, along with sodium metavanadate, obviating toxicity symptoms without eliminating diabetic symptom alleviation [190]. A third, and more recent method, was enteric encapsulation of vanadyl sulfate, which enhanced bioavailability almost twofold, extended the mean residence time in the blood to 11.7 h, and passed through the stomach without disintegration, thus minimizing gastrointestinal irritation [191]. Lastly, transdermal delivery (via patch) has been tried both passively and by iontophoresis, using peroxovanadium, with and without complexation by 1,10-phenanthroline, in diabetic rats [192,193]. Increased blood levels of vanadium were achievable by this method; however, blood glucose reduction was modest. To our knowledge, none of these proposed methods of alternative vanadyl ion delivery is in current development.

6. Phase I human clinical trial of BEOV

And so, 100 years after 'Phase 0' clinical trials of vanadium for diabetes, a Phase I clinical trial of an oxovanadium(IV) complex was carried out by Medeval Ltd in Manchester, UK. The first human clinical trial using the ethylmaltolato vanadium coordination complex, BEOV, was completed in 2000 (first reported at ICBIC-11, in Cairns, QLD, Australia, July, 2003 [3]). The overall objective of this trial was to assess the safety and tolerability of BEOV. Specific objectives were initially to: (1) assess the safety and tolerability of single, escalating dose of orally administered BEOV; (2) determine the pharmacokinetics of modest doses of BEOV from measured plasma, urinary and fecal $[V]_{total}$; and (3) compare the bioavailability of a well-tolerated dose of oral BEOV and an equivalent molar dose of oral $VOSO_4$ [4]. Volunteers (40, in total) were healthy (not diabetic) male, or surgically sterile females between the ages of 18 and 45 years of age, within 20% of normal weight and able to provide informed consent.

Initially, four volunteers were given a single 10 mg oral dose, open label. This was followed by an escalating dose study, with four dose levels: 25, 35, 60, 90 mg BEOV, four subjects at each dose level and two placebo controls. The third stage was a bioavailability study, in which four volunteers were each given a single 50 mg dose $VOSO_4$, open label. A last phase, appended as a modification of the original study, looked at the effects of fasting or feeding on bioavailability. In this phase, eight subjects were each given two single 75 mg doses of BEOV, one dose fasted, one fed (non-fasted) in an open-label, randomized, cross-over design. Summary results for stages two and three of the study shown in Table 1 are for volunteers given either BEOV or $VOSO_4$.

No adverse health affects were observed in any of the volunteers. Gastrointestinal, liver and kidney function, and blood parameters all remained within normal levels throughout the study. Pharmacokinetic analysis results (Table 1) showed a clear dependence (non-proportional) of area under the curve (AUC) and proportion of administered dose (%AD) in urine on dose, but time to maximal concentration (T_{max}), time to disappearance of 50% of the prodrug dose ($t_{1/2}$) and renal clearance rate were all independent of dose. Note that the 60 mg dose of BEOV (0.17 mM) is closest to the 50 mg dose of $VOSO_4$ (0.23 mM), in terms of vanadium dose administered.

The total recovery of vanadium over the 72 h collection period (Table 1), as a percentage of the administered dose (%AD), was less than 100% in all except the smallest dose, indicating probable continued tissue accumulation, most likely in bone. Vanadium from $VOSO_4$ was absorbed more slowly (based on the time to maximal concentration T_{max}), achieved a much lower maximal systemic concentration (C_{max}), a lower %AD in urine and had a slower renal clearance, compared to vanadium derived from BEOV. T_{max}, half-life ($t_{1/2}$) and renal clearance rate (Cl_R) were all independent of dose. C_{max}, AUC and urinary vanadium as a percent of dose all increased non-proportionally with dose; fecal vanadium decreased non-proportionally with dose. The pharmacokinetic advantage established for BEOV in this study can be expected to apply to BMOV as well [109].

Feeding had a very large negative effect on availability of BEOV (Table 2), suggesting ligand substitution by foodstuff components. Oral availability of BEOV might thus be improved by administration prior to meals, in order to avoid this effect. The relative bioavailability of vanadium from BEOV was calculated relative to vanadyl sulfate as $((AUC/Dose)_{BEOV} - (AUC/Dose)_{VOSO_4}) \times 100$. Thus, bioavailability was estimated to be three times that of an equivalent dose of vanadium from $VOSO_4$, corroborating earlier results in experimental animals [101,104]. However, vanadium absorption after administration of 75 mg BEOV in the fasted state was approximately 13 times higher than from administration of the same dose in the fed state. Tolerability was comparable in both fed and fasted states, with no clinically significant adverse events or changes in safety parameters assessed.

Table 1
Pharmacokinetic assay results of Phase I clinical trial of KP102 (BEOV)

Parameter	BEOV					50 mg $VOSO_4$
	10 mg	25 mg	35 mg	60 mg	90 mg	
T_{max} (h)[a]	3.1	3.5	3.0	3.5	0.8	6.0
C_{max} (ng/mL)[b]	4.1	38.5	28.7	65.0	170.0	21.6
AUC (ng h/mL)[c]	276	1392	1273	3286	8719	1136
$t_{1/2}$ (h)[d]	63.5	45.1	55.1	52.5	61.0	59.2
% $AD_{urine\ (0–72\ h)}$[e]	1.90	5.81	3.11	4.64	11.33	1.04
% $AD_{feces\ (0–72\ h)}$	139.8	66.8	40.5	73.8	57.4	40.4
Cl_R (L/h)[f]	0.237	0.168	0.226	0.204	0.243	0.142

Escalating doses of BEOV, or a single dose of vanadyl sulfate ($VOSO_4$), were administered orally to six groups of four volunteers each.

[a] T_{max}: time to maximal concentration of vanadium (V).

[b] C_{max}: maximal concentration of V.

[c] AUC: area under the curve of V disappearance from plasma ([V] vs. time).

[d] $t_{1/2}$: half-life of V persistence in plasma.

[e] % AD: percentage of the administered dose of V.

[f] Cl_R: renal clearance of V, estimated from total V excreted in urine over the 72 h collection period (Ae_u) and AUC: $(Ae_u/AUC) \times 100$.

Table 2
Comparison of fed versus fasted parameters for BEOV absorption

Parameter	Fasted	Fed	P values*
T_{max} (h)[a]	0.75	4.00	0.06
C_{max} (ng/mL)[b]	426.1	34.0	0.0001
AUC (ng h/mL)[c]	28,777	2353	0.0001
$t_{1/2}$ (h)[d]	76.83	108.25	NS
% $AD_{urine\ (0–169\ h)}$[e]	36.1	2.3	–
% $AD_{feces\ (0–168\ h)}$	17.5	108.8	–
Cl_R (L/h)[f]	0.174	0.166	NS

Eight volunteers received two single doses of 75 mg (11.1 mg V) dose of BEOV on separate occasions, either fasted (overnight and at least 4 h after dosing) or fed (standard breakfast immediately after dosing), separated by approximately 2 weeks.

* Analysis of variance (ANOVA) was performed using the GLM procedure in SAS (version 6.12).

[a] T_{max}: time to maximal concentration of vanadium (V).

[b] C_{max}: maximal concentration of V.

[c] AUC: area under the curve of V disappearance from plasma ([V] vs. time).

[d] $t_{1/2}$: half-life of V persistence in plasma.

[e] % AD: percentage of the administered dose of V.

[f] Cl_R: renal clearance of V, estimated from total V excreted in urine over the 168 h collection period (Ae_u) and AUC: $(Ae_u/AUC) \times 100$.

7. Summary and conclusions

Vanadium compounds for treatment of diabetes are somewhat enigmatic: the therapeutic potential is clearly there, but the mechanisms, and a consistent dosing regimen, are hard to pin down. Efforts to identify a particular ''best'' vanadium-based insulin enhancing agent are unlikely to yield a unique candidate, as biomolecular transformation *in vivo* is a necessary feature of vanadium's *modus operandi*. That said, one can still aim to choose ligands for vanadyl complexation such that premature redox conversion is inhibited, transferrin and at least some albumin, binding are favored, safety of the dissociated ligand is assured, and synergistic effects are taken advantage of, where possible. As more details of vanadium's biodistribution *in vivo* are forthcoming, individualized treatment regimens may become possible, hence optimizing the performance of any given vanadium pharmaceutical agent.

8. Abbreviations

acac	acetylacetonate
AD	absorbed dose
ADME	absorption, distribution, metabolism and excretion
Ae_u	total urinary excretion [for a specific metal ion, in a specific time period]
apoTf	apo-transferrin
AUC	area under the curve
BEOV	bis(ethylmaltolato)oxovanadium(IV)
BMOV	bis(maltolato)oxovanadium(IV)
Cl_R	renal clearance
C_{max}	maximal concentration
EPR	electron paramagnetic resonance
Habtd	((±)-5-{4-[(5-hydroxy-4-oxo-4H-pyran-2-ylmethyl)amino]benzyl}thiazolidine-2,4-dione
Hallix	allixin
Hbetd	((±)-5-[4-(5-hydroxy-4-oxo-4H-pyran-2-ylmethoxy)benzylidene]thiazolidine-2,4-dione
Hbtd	((±)-5-[4-(5-hydroxy-4-oxo-4Hpyran-2-ylmethoxy)benzyl]thiazolidine-2,4-dione
Hema	ethylmaltol
Hiodopic	iodopicolinic acid
Hma	maltol
Hmpic	6-methylpicolinic acid
Hmetf	metformin
Hpic	picolinic acid
HSA	human serum albumin
LD_{50}	dose at which 50% of experimental animals die
MIF	metal inhibitory factor
6MPA	6-methylpicolinato
pic	picolinato
PTPase	protein tyrosine phosphatase
i.p.	intraperitoneal
STZ	streptozotocin
T_{max}	time to maximal concentration

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