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Vanadium Compounds in the Treatment of Diabetes

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1. INTRODUCTION

In the years since the publication of our last review in this series, "Vanadium Compounds as Insulin Mimics" [1], we have observed an exponential growth in both the interest in this topic, and the number of vanadium-based drug candidates designed and promulgated for the treatment of diabetes mellitus. In 1995, there were fewer than a dozen compounds being given serious consideration; now at least ten times that many are being investigated. As this topic has expanded, many technical reviews and summary research reports have been published [2-7]. Herein we present a less technical overview and discussion of clinical implications of the research.

Vanadium compounds, given orally, can counteract the high blood sugar and insulin resistance of diabetes mellitus as it commonly presents. This much has been well established by numerous trials and investigations ([8] and references therein). Certainly the need for orally available hypoglycemic agents is burgeoning, with the incidence of diabetes currently estimated at 6% of the populations of developed countries, and alarming increases in many populations of underdeveloped nations [9]. Insulin, the treatment of first choice but last resort for all types of diabetes, is not orally available. Oral hypoglycemic treatments, such as sulfonylureas, biguanides and thiazolidinediones, often require combination treatment to lessen the side effects and to reduce resistance with increasing length of use [9].

With almost twenty years of *in vivo* evidence that vanadium compounds can be effective antidiabetic agents, what are the outstanding obstacles to their clinical use? Primarily, as with any number of metal-based therapeutic agents, the perception of toxicity as an insurmountable barrier has constrained research investigations [10,11]. Secondly, a multifaceted mechanism of action, rather than an identifiable specific molecular target, discourages pharmaceutical development [10]. Despite these drawbacks, vanadium compounds as insulin enhancing agents are being developed widely [2-5]. Improved understanding of the metabolic pathways of their utilization, along with greatly increased understanding of insulin's mechanism of action, will undoubtedly lead to clinically useful formulations.

Interest in vanadium as a possibly essential metal ion and/or as a clinically useful adjunct dates back many decades. Schroeder's statement forty years ago that "No other trace metal has so long had so many supposed biological activities without having been proven to be essential" [12] is even truer today. Indeed, human clinical trials of vanadium for medical use date back more than fifty years, with early reports of cholesterol-lowering efficacy in non-diabetic subjects [13-15], and even a century old anecdotal report of improvement in glucose tolerance in diabetic patients [16]. Vanadium, contained in a salt or bound to an organic ligand, cannot entirely substitute for insulin [6]. Therefore, the majority of investigations now concentrate on vanadium's potential as a treatment for type 2 diabetes mellitus (T2DM), by far the most common type of diabetes mellitus [17].

2. INSULIN RESISTANCE

2.1. Type 2 Diabetes

Type 2 diabetes mellitus is a metabolic disorder defined by abnormally high blood glucose concentration and a characteristically slow response to an oral glucose challenge [17]. Typically, the serum insulin level is not deficient, and may even be above normal, creating its own set of

problems and contributing to overall mortality [9]. This type of diabetes was formerly considered a disease of the elderly, linked to hypertension and obesity; often it was referred to as "maturity-onset diabetes", or noninsulin-dependent diabetes (NIDDM). Now, as many younger patients – adolescents and even children – are being diagnosed as type 2 diabetic [18], clinicians are more likely to focus on insulin insensitivity as the key to the disorder [17]. Defects in intracellular signaling are associated with the abnormalities of glucose, lipid and insulin metabolism, with peripheral insulin resistance appearing first in skeletal muscle [9,19]. Diabetes mellitus has a multi-factorial origin: both genetic and environmental influences have been implicated.

In the early stages of type 2 diabetes, pancreatic insulin secretion may increase sufficiently to maintain normal plasma glucose, even as over-consumption leading to obesity is straining metabolic resources [17]. Hyperglycemia (high blood sugar) starts to appear when insulin production can no longer keep up. Insulin resistance in adipose and hepatic tissues also becomes evident, and increased free fatty acids (FFA's) further exacerbate the problem, with concurrent defects in several organs all contributing to the insulin resistance syndrome [20,21].

2.2. Animal Models of Type 2 Diabetes

Recent studies have focused on defects in the insulin signaling pathways in the pathophysiology of type 2 diabetes mellitus [19,21]. Important keys to the intricately interwoven pathways have been discovered by observing metabolic variations in a host of different knock-out mouse models [17]. These animal models have targeted specific deletions in the many components of the insulin signaling cascade, such that particular components of regulatory pathways can be pinpointed and examined. Genetic factors that can influence insulin sensitivity may be at the level of insulin receptors, intracellular enzymes or multiple glucose transporters [22].

Whereas such models are of intense interest in teasing apart the complex machinery of hormonal influences on glucose and lipid metabolism, they are not particularly well-suited to evaluating the relative merits of alternative insulin mimics or enhancers. For this purpose, either inexpensive chemically-induced diabetic rats (especially streptozotocin (STZ)-diabetic rats) [23], or spontaneously diabetic murine models are favored, e.g., *db/db* mice [24], the BB rat [25] *fa/fa* rats [26], KKA^y mice [27], and Zucker diabetic fatty (ZDF) rats [28].

All animal models of diabetes are at best partially successful in replicating the full panoply of disease symptoms in either type of diabetes. STZ functions by destroying the insulin secreting β-cells of the pancreas, thus drastically reducing pancreatic insulin secretory capacity. Diabetic characteristics (low plasma insulin; high plasma and urinary glucose; high plasma lipid levels) follow soon after injection with STZ [23,29]. Models of T2DM replicate the disorder to varying degrees. Glucose intolerance combined with obesity characterizes *db/db* mice [24]. The original *fa/fa* Zucker rat [26] was more a model of obesity, with mild glucose intolerance; however a mutation, the Zucker diabetic fatty rat, is obese *and* glucose intolerant, and currently one of the best models available of T2DM [28]. The KKA^y mouse is a model of genetically induced T2DM that develops early obesity and hypoglycemia [27].

2.3. Oral Treatment Modalities in Humans

Because diabetes mellitus is a highly heterogeneous disorder, effective treatment varies widely from patient to patient. A rare few are able to control the disease by diet and exercise alone; most require some form of oral hypoglycemic agent or combination of same [9]; and many eventually require insulin by injection as well; despite the moniker, non-insulin dependent diabetes mellitus is basically a misnomer.

Introduction of a systematic means of monitoring diabetic control, hemoglobin A_{1c} (HbA_{1c}) [30] ushered in a new era of diabetes treatment. Previously, use of fasting blood glucose in combination with frequent oral glucose tolerance testing and various subjective measures was the only way to judge efficacy of chronic treatment regimens [31]. Advocacy of 'tight glycemic control' was then highly controversial, as its utility could not be proven [32]. With the completion of the Diabetes Control and Complications Trial (DCCT) [33], this issue was finally resolved, as maintenance of low HbA_{1c} scores (an integrated measure of blood glucose levels over a several month period) was shown to correlate positively with delayed onset (and reduced severity) of secondary complications of dia-

betes, such as retinopathy, neuropathy, nephropathy, and atherosclerotic disease [34].

An even more recent change has been the increased availability of oral hypoglycemic agents [9], of which there are now five distinct classes. None are free of side effects, nor are they consistently effective with prolonged use; however, they do offer a range of mechanisms of action, and are often used in combination with each other, or with insulin (which requires injection). The five categories of antihyperglycemics, most introduced just in the last six years, include sulfonylureas [35], biguanides (e.g., metformin) [36], thiazolidinediones (e.g., pioglitazone) [37], α-glucosidase inhibitors (AGI's, e.g., acarbose) [38] and non-sulfonylurea insulin secretagogues (e.g., nateglinide) [39]. Sulfonylureas constitute the oldest category, introduced almost fifty years ago [35]; second generation sulfonylureas include glyburide, glipizide and glimepride [40].

Each of the first four classes targets a different tissue: sulfonylureas facilitate insulin release from the pancreatic β-cells; biguanides decrease hepatic glucose production; thiazolidinediones decrease lipolysis, lower circulating free fatty acids and increase skeletal muscle glucose uptake; and α-glucosidase inhibitors delay intestinal carbohydrate absorption [9]. Non-sulfonylureas act similarly to sulfonylureas, indirectly stimulating insulin secretion at the level of the β-cell [39].

All except the AGI's are approximately equally effective, reducing HbA_{1c} by 1 – 2%; AGI's are about half as effective; however they are less likely than the others to result in hypoglycemia or weight gain [9]. All types, especially the sulfonylureas, suffer from declining efficacy the longer they are used. Primary care physicians thus need to constantly monitor and adjust the type, combination, and dosage of these drugs. Clearly, ideal treatment modalities for T2DM dictate further refinements.

3. VANADIUM COMPOUNDS AS INSULIN MIMETIC AGENTS

3.1. History: *In vitro* and *in vivo* Insulin-like Effects of the Vanadium Salts Vanadate (VO_4^{3-}) and Vanadyl (VO^{2+})

The serendipitous discovery of vanadium's potent inhibition of ATPases [41] led shortly thereafter to discovery of its *in vitro* effects on glucose

metabolism, e.g., stimulation of glucose uptake and concomitant inhibition of lipolysis [42]. Reference to vanadium's potential use as an insulin mimic first appeared in print in 1980 [43,44]. Similarity between vanadium and ouabain in terms of activation of glucose transport and oxidation in adipocytes was attributed to inhibition of Na^+,K^+-ATPase, or the sodium pump, suggesting that these compounds might be useful in elucidating insulin action on target cells. Five years later, the first report appeared of *in vivo* insulin-enhancing effects of orally administered vanadium [45]. Not only were plasma glucose and lipid levels lowered, but also thyroid hormone levels were corrected and diabetes-associated cardiomyopathy prevented in STZ-diabetic rats [45].

The multiplicity of vanadium's effects (often as vanadate) that 'mimic' those of insulin is impressive: both vanadium and insulin stimulate glucose uptake and glucose oxidation in adipocytes, glycogen synthesis in diaphragm and liver cell homogenates [42-44]. Both inhibit gluconeogenesis in liver, glucose transport in intestinal cells and lipolysis in fat cells [46,47]. Millimolar concentrations of sodium vanadate added to various tissue homogenates, including brain, skeletal muscle and intestinal cells, result in enhanced glucose uptake and transport [48,49]. Despite the similarity of effect, there is no specific evidence that insulin and vanadium act through a common mechanism. On the contrary, the mechanism of action of vanadium has been proposed as one that bypasses the insulin receptor [50], and is most likely closely linked to its potent inhibition of protein tyrosine phosphatases (PTPases), perhaps at more than one locus (there are many) in the insulin regulatory cascade [19,51].

In vivo, at doses that commonly result in micromolar concentrations in the bloodstream, vanadate and vanadyl have been shown repeatedly to counteract both the hyperglycemia and hyperlipidemia of diabetes, in models of both type 1 and type 2 diabetes ([52] and references therein). Secondary complications, such as diabetic cardiomyopathy and onset of cataracts, have also been alleviated by orally administered vanadium compounds [53,54].

In the *fa/fa* Zucker rat, which is hyperinsulinemic and obese, sodium orthovanadate in drinking water and food pellets (~0.5 mmol V kg^{-1} day^{-1} overall) reduced food and fluid intake, decreased weight gain, lowered hyperinsulinemia towards normal levels, and improved glucose tolerance,

whereas pair feeding (a system of obviating the effects of reduced food intake in a test group by feeding the same amount to weight-matched animals of the control group) only partially reversed these parameters [55]. In a subsequent study using the same rat strain and protocol, sodium orthovanadate improved glucose utilization without increasing glucose transporter levels in muscle [56]. *Ob/ob* mice are obese and glucose intolerant as a result of hypersecretion of insulin from the beta cells of the pancreas. In this strain of mouse, plasma glucose and insulin were reduced by 50% within one week of beginning treatment with sodium orthovanadate, 1.6 mM in drinking solutions [57]. Pancreatic insulin stores quadrupled in the vanadium-treated, vs. control, *ob/ob* mice. Glucose tolerance of *db/db* mice (obese, glucose intolerant) was also improved by inclusion of sodium orthovanadate (0.5 mmol V kg^{-1} day^{-1}) in the feed [58]. No effects on plasma lipids were observed in any of these models of type 2 diabetes. Even though correction of hypercholesterolemia and hypertriglyceridemia by vanadium treatment is commonly observed in models of type 1 diabetes, such as STZ-rats [59] and alloxan-diabetic rats [60], it is usually not seen in genetically-diabetic, type 2 murine models. Nonetheless, the fact that vanadium enhances insulin function without increasing the plasma levels of insulin has particular relevance to type 2 diabetes, wherein hyperinsulinemia is a frequent pathophysiological determinant of disease progression [61].

In humans, clinical trials of both vanadate and vanadyl have been carried out in either or both type 1 and type 2 diabetic subjects. Modest improvements in glucose tolerance and/or insulin sensitivity, especially in type 2 diabetes, have been observed, although clinical trials to date have only been carried out for short periods [62-67]. Treatment with sodium metavanadate (1 mmol day^{-1}) for 2 weeks resulted in significantly improved insulin sensitivities as measured by a two-step euglycemic hyperinsulinemic clamp technique in T2DM subjects only [62]. All subjects had decreased cholesterol levels, and type 1 diabetic subjects had decreased insulin requirements during the treatment period. HbA_{1c} levels were decreased 10% on average for both groups.

Oral vanadyl sulfate (0.5 mmol day^{-1}) for 3 weeks followed by 2 weeks of placebo demonstrated increased insulin sensitivity in T2DM subjects, sustained during the withdrawal period [63]. Decreases in fasting plasma glucose (FPG) and $\%HbA_{1c}$ were observed. Safety and efficacy were

tested at a higher dose of vanadyl sulfate therapy for 4 weeks, 1 mmol day^{-1}, resulting in significantly decreased FPG by 20%, and decreased hepatic insulin resistance [64]. This dose was fairly well tolerated (6 of 8 had gastrointestinal disturbance, but this resolved over the course of the study), and was not anorexigenic. Subsequently, 16 T2DM patients were given three graded doses of vanadyl sulfate (75, 150, and 300 mg $VOSO_4$ day^{-1}, equivalent to 0.35, 0.70, and 1.4 mmol V day^{-1}) over a period of 6 weeks [64]. Again, both FPG and $\%HbA_{1c}$ decreased significantly (though not at the lowest dose); however there was no correlation between plasma vanadium and clinical response. Vanadium is a redox active element; nonetheless, oxidative stress was not increased overall with vanadium treatment, either in experimental animals or in humans [54,64].

A 12-week study in weight-training (non-diabetic) adults, vanadyl sulfate, 0.5 mg kg^{-1} day^{-1} (~0.7 mmol V day^{-1}), demonstrated a complete lack of toxic, anabolic, or hematologic effect [66]. Most recently, 11 T2DM subjects were treated with 0.7 mmol day^{-1} vanadyl sulfate (150 mg day^{-1}) for 6 weeks, and had 20% reduced fasting plasma glucose, 10% reduced $\%HbA_{1c}$ (from 8.2 ± 0.4 to 7.6 ± 0.4%) and decreased fructosamine (a measure of advanced glycosylation endproducts [34]) levels by 16% [67]. Vanadyl sulfate treatment also lowered plasma total cholesterol and low density lipoprotein (LDL) cholesterol, but did not affect 24-hour ambulatory blood pressure, and was well-tolerated with a progressively increased dose treatment regimen.

3.2. Vanadyl Bis(ligand) Complexes

Numerous small ligands have been used to increase the potency of vanadium, to decrease the dose required to achieve normoglycemia, and to lessen the likelihood of exceeding the toxic threshold for vanadium [68]. Prominent among these are $V^{IV}OL_2$ type compounds, wherein L is a bidentate monoprotic ligand.

3.2.1. Variations on the Maltol Theme

Maltol, a 3-hydroxy-4-pyrone (Hma in Figure 1), has proven to be an outstanding first choice for a ligand designed to further enhance the

insulin-like properties of the oxovanadium(IV) ion. It has a very low toxicity profile and is an approved food additive in the US, UK and Canada [69]. It forms thermodynamically stable, neutrally-charged complexes that have a bio-compatible hydrophilic/lipophilic balance. Its effectiveness as an absorption-promoting ligand has been proven with a variety of other metals, including Al^{3+}, Ga^{3+}, In^{3+}, Mo^{6+} and Fe^{3+} [70-73]. With vanadyl ($[VO]^{2+}$), maltol readily forms bis(maltolato)oxovanadium(IV), usually referred to by its acronym, BMOV. Since its initial study more than a decade ago, BMOV has become the most widely and intensively tested of a whole series of $V^{IV}OL_2$ complexes proposed as insulin enhancing compounds, where L is either a close *or* distant analogue of maltol [74-78].

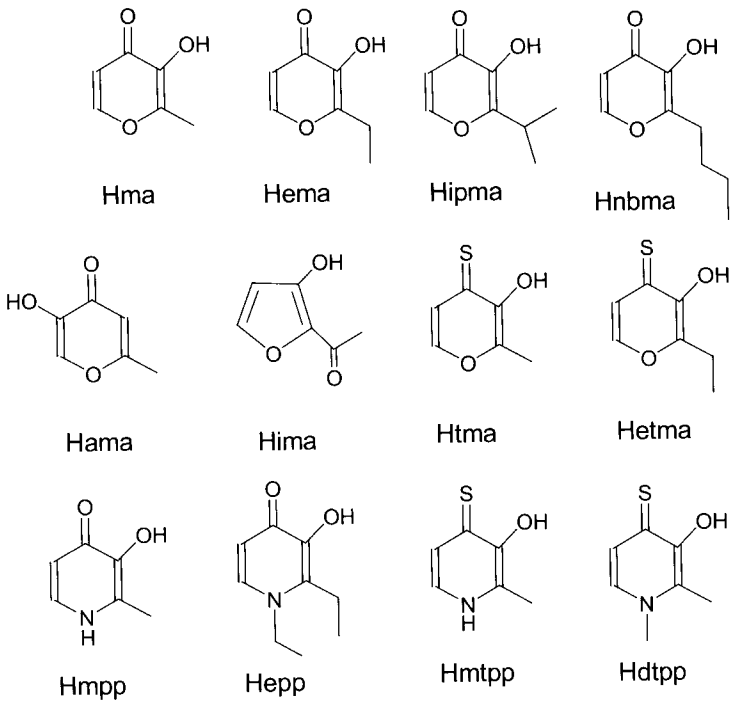


FIG. 1. Variations on the maltol (Hma) theme. A series of alkyl maltol, alkyl hydroxypyridinone, and structural analogues used as ligands for vanadyl ions in candidate insulin enhancing agents.

BMOV lowers plasma glucose, cholesterol and triglycerides, improves cardiac function and has no effect on plasma insulin levels in STZ-diabetic rats [74-80]. In *fa/fa* rats, BMOV significantly improves insulin secretory function and ameliorates glucose intolerance [79]. In ZDF rats, BMOV (0.4-0.8 mmol kg^{-1}) decreased plasma glucose, insulin and triglyceride levels [80]. Bis(ethylmaltolato)oxovanadium(IV) (BEOV) [77a] has actually completed phase 1 clinical trials in humans.

TABLE 1

Bis(ligand)alkylmaltol oxovanadium(IV) compounds ranked according to efficacy at three critical time points in an acute screening trial in STZ-diabetic rats[a]

12 h post		24 h post		48 h post	
	%PG		%PG		%PG
$VO(ima)_2$	45	$VO(ma)_2$	54	$VO(ipma)_2$	56
$VO(ma)_2$	43	$VO(ipma)_2$	51	$VO(ma)_2$	47
$VO(ema)_2$	41	$VO(ima)_2$	50	$VO(ema)_2$	29
$VO(ipma)_2$	31	$VO(ema)_2$	43	$VO(ima)_2$	26
$VO(etma)_2$	20	$VO(etma)_2$	18	$VO(dtpp)_2$	16
$VO(tma)_2$	17	$VO(tma)_2$	18	$VO(etma)_2$	13
$VO(mtpp)_2$	13	$VO(mtpp)_2$	15	$VO(mtpp)_2$	11
$VO(dtpp)_2$	–9	$VO(dtpp)_2$	7	$VO(tma)_2$	–2

$\%PG = (PG_{control}\text{-}PG_{test})/PG_{control} \times 100\%$.

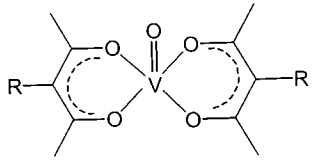
[a] Plasma glucose-lowering at 12, 24, and 48 hours after oral gavage or i.p. injection (ED_{50} dose, 0.6 mmol kg^{-1} oral, 0.1 mmol kg^{-1} i.p.). By comparison, $VOSO_4$ lowered plasma glucose 17% at 12 h, 12% at 24 h and -3% at 48 h at the same dose in STZ-diabetic rats.

Many variations on the maltol (Hma) theme have been synthesized and characterized (Figure 1). The top row ligands are all alkylmaltols with alkyl chains of varying length and branching. Vanadyl compounds with Hma, Hema, and Hipma all have similar efficacy in STZ-diabetic rats

[77a], as do vanadyl bisligand complexes with the isomeric conversion products, Hama and Hima, allomaltol and isomaltol, respectively (Table 1) [77b]. Sulfur substitution of the pyrone ring oxygen (as in Htma and Hetma) eliminated efficacy in one-dose screening trials [77b], but not in longer assays, either in *ob/ob* mice or in STZ-diabetic rats [81]. Nitrogen substitution in the maltolato heterocycle (Hmpp and Hepp) reduced but did not eliminate glucose-lowering efficacy in short-term STZ-diabetic rat trials, but did significantly reduce solubility, potentially a problem for oral administration [82a]. These ligands formed V(III) complexes preferentially (*vide infra*) and were also not very stable. Nitrogen substitution in the maltolato heterocycle combined with sulfur-substitution for oxygen (Hmtpp and Hdtpp) produced compounds that were *less* effective than vanadyl sulfate and also insoluble [77c]. Table 1 demonstrates the range of efficacies possible within the bis(ligand)alkylmaltol series of vanadyl complexes.

3.2.2. *Acetylacetonates and Picolinates*

Bis(2,4-pentanedionato-*O,O*)oxovanadium(IV), $VO(acac)_2$ (below), was, to our knowledge, first reported nearly a century ago [83]; however it, and several analogues have recently been considered as potential insulin mimetic agents [84,85]. Both the 3-methyl- and 3-ethyl-2,4-pentanedionato vanadyl complexes, $VO(Me\text{-}acac)_2$ (R = CH_3) and $VO(Et\text{-}acac)_2$ (R = C_2H_5), respectively, have been tested biologically [84-86].



R = H, $VO(acac)_2$
= CH_3, $VO(Me\text{-}acac)_2$
= C_2H_5, $VO(Et\text{-}acac)_2$

In an *in vitro* study, $VO(acac)_2$, 5-100 μM, was more effective than vanadyl sulfate ($VOSO_4$) in stimulating lipogenesis in isolated fat cells, and had identical effectiveness in stimulating activity of a cytosolic protein kinase (CytPTK) [86]. Intraperitoneal injection (25 μmol kg^{-1}) of $VO(acac)_2$ slightly lowered plasma glucose levels in STZ-diabetic rats; $VO(Et\text{-}acac)_2$ at the same dose was ineffective [85]. BMOV, $VO(acac)_2$,

and $VO(Et\text{-}acac)_2$, 0.4 mM orally, were compared. All were mildly effective as oral glucose-lowering agents, significantly more effective than $VOSO_4$ at the same dose. The ratio of vanadium intake to plasma vanadium was higher for $VOSO_4$ than for BMOV, $VO(acac)_2$ and $VO(Et\text{-}acac)_2$, all of which were indistinguishable [85]. The mechanism of insulin mimesis by $VO(acac)_2$ is thought to be closely tied to its aqueous stabilization by adduct formation with serum albumin, and possible interactions with other serum proteins [87].

Other compounds of the form $V^{IV}OL_2$ have also proven effective in modifying insulin activity *in vivo*. Bis(pyridine-2-carboxylato)oxovanadium(IV), vanadyl picolinate (VOPIC) has been tested in several laboratories [88-90]. The insulin enhancing effects of vanadyl picolinate appear to be dependent on dose as well as delivery method. VOPIC, 0.2 mmol kg^{-1} orally for 2 days, then 0.1 mmol kg^{-1} for 11 days, normalized plasma glucose in STZ-diabetic rats [89]. Plasma insulin levels increased significantly during this trial [89]. VOPIC, 2.4 mM in the drinking water (~1.0 mmol kg^{-1} day^{-1}) lowered plasma glucose without increasing insulin levels in STZ-diabetic rats, but was accompanied by gastrointestinal (GI) irritation [89]. Intraperitoneal (i.p.) administration of VOPIC, 0.2, 0.1, and 0.06 mmol V kg^{-1} day^{-1} [89,90], also lowered plasma glucose levels, but with increased bilirubin at the highest dose. In comparison to its methylpicolinate analogue, VOMPA, the picolinate complex had a less sustained response and was less effective as an inhibitor of FFA release *in vitro* [2].

3.3. V(III) Agents

Mononuclear coordination complexes of V(III) are not commonly considered for therapeutic applications: they oxidize rapidly to V(IV) and/or V(V) in aqueous solutions at pH >3 [3,91]. Nonetheless, complexes of V(III) with maltol (and analogues) have proven to have insulin enhancing activity, despite the fact that prior art teaches against it [82a]. V(III) complexes with maltol and analogues are hydrolytically and thermodynamically stable; some (e.g., $V(ma)_3$) are also reasonably air stable, although this is unusual for V(III) chelates of this type [82a]. Treatment with $V(ma)_3$, and to a lesser extent, $V(ema)_3$, $V(ama)_3$ and $V(ima)_3$ compared to BMOV demonstrated glucose lowering in STZ-diabetic rats, whether

administered by i.p. injection or orally, with no significant toxicity, and no fatalities, but with less sustained insulin enhancement, and a lower percent glucose-lowering overall [82b].

3.4. Oxovanadium(V) Complexes and Anions as Insulin Mimetic Agents

A V(V) analogue of $VO(pic)_2$, $NH_4[VO_2(pic)_2]$, has also been given consideration as an insulin mimetic agent [92,93]. $[VO_2(pic)_2]^-$ is likely to be intracellularly transported via anionic transporters, or via protonation to a neutral compound, which then could be absorbed by passive diffusion. *In vivo* testing has included oral administration in drinking water, average dose 0.15 mM V kg^{-1} day^{-1}, to diabetic cats (n = 5) for 16 weeks resulting in 50% glucose lowering and significant lowering of fructosamine levels as well [93]. Polydipsia, polyuria, weight loss and polyphagia resolved during the experimental period, in treated animals, compared to placebo (n = 6) [93].

Using *in vitro* assays, glucose uptake by Simian virus transformed Swiss 3T3 mouse fibroblasts (SV 3T3 cells) and by nontransformed human fibroblasts (F26), a series of V(IV)- and V(V)-containing compounds have recently been compared [4]. Compounds were also compared for relative toxicity by the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). The results were not entirely consistent. For example, a V(V) dipicolinato complex, effective as a glucose-lowering agent in diabetic cats [5,93], showed evidence of high efficacy in the SV 3T3 fibroblasts, but not in stimulating glucose uptake in the F26 cell line [4]. The extent of biological transformation among these complexes was unclear; however, a curious result was the lack of any clearly distinguishable differences between V(IV) and V(V) compounds in terms of relative efficacy over a wide range of coordinating ligands [4]. Overall, V(IV) complexes tended to be more efficacious compared to those of V(V) as insulin mimetics according to the SV3T3 glucose uptake assay, but were also judged to be more toxic based on results of the MTT assay. The former conclusion corroborates *in vivo* studies with V(IV) and V(V) compounds [76,94]; the latter is inconsistent with previous *in vivo* toxicological evaluations [95,96].

3.5. Mono- and Di-peroxovanadates

Vanadium(V) in the presence of hydrogen peroxide tends to form peroxovanadates, coordination complexes containing a $[VO(O_2)_{(n=1,2)}]$ moiety. First discovered as mixtures and subsequently purified to homogeneity, peroxovanadates are several orders of magnitude more potent than vanadyl or vanadate complexes *in vitro* as PTPase inhibitors [97-99]. The mechanism of action of peroxovanadates has been established as irreversible enzymatic inhibition, with increased oxidative stress an important contributing factor [100]. Because intracellular hydrogen peroxide levels are known to increase with diabetes [101], a possible mechanism for vanadium's insulin mimesis has even been suggested to be *in situ* formation of peroxovanadates with consequent PTPase inhibition [102]. Although it seems unlikely that this is the whole story, due to the many other known *in vitro* and *in vivo* effects of vanadium salts and compounds, the fact of very potent PTPase inhibition remains. Hence, finding a stable peroxovanadate for use as an antidiabetic agent presented an attractive possibility [98,99,103-106].

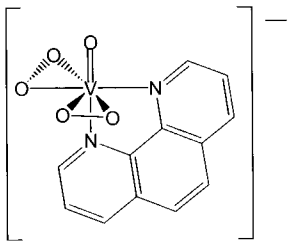


FIG. 2. Structure of [bpV(phen)] (oxodiperoxo(1,10-phenanthroline)vanadate(V) monoanion).

Screening a series of well-characterized mono- and di-peroxovanadate compounds of the general type $[VO(O_2)_x(L\text{-}L')]^-$, where x = 1 or 2, and L = a bidentate ligand, such as picolinate or 1,10-phenanthroline, isolated as NH_4^+ or K^+ salts for *in vitro* activation of insulin receptor kinase and inhibition of PTPase, demonstrated that minor modifications in the ligand could result in major differences in efficacy [98]. Several

compounds, especially picolinatooxoperoxovanadate [mpV(pic)] and pyridine-2,6-dicarboxylatooxoperoxovanadate [mpV(2,6-pdc)] showed promise [98]. Potassium oxodiperoxo(1,10-phenanthroline)vanadate(V) trihydrate $[bpV(phen)]^-$ (Figure 2) was also a potent *in vitro* PTPase inhibitor, and had the additional advantage of being reasonably stable thermodynamically.

These compounds were subsequently tested *in vivo*, both in STZ-diabetic and in spontaneously diabetic BB rats. For mpV(pic), the lowest effective dose (LED) was determined to be 0.4 μmol kg^{-1}, while the lowest dose producing mortality was 15 times higher. By contrast, mpV(2,6-pdc) had an LED = 24 μmol kg^{-1} which was twice the lowest dose producing mortality [99]. At LED's of 0.75-6.0 μmol V kg^{-1}, bpV(pic), bpV(phen) and bpV(Me_2phen) lowered plasma glucose in BB rats, whether given i.v., i.p., or subcutaneously (s.c.) [99]. (Toxic doses for these compounds were determined as >12, >24, and >48 μmol V kg^{-1}, respectively [99]). A major challenge for continuing development of peroxovanadates is lack of oral bioavailability [103-105].

The coordination of vanadium(V) to imidazole presents structural analogies to the coordination of vanadium to histidine residues in vanadium-containing haloperoxidases and some phosphorylases. A model of this interaction, an imidazole peroxovanadium complex [106] has been shown to enhance insulin receptor autophosphorylation in human liver cell culture, as well as to increase glucose transport in rat adipocytes, at concentrations ranging from 1 μM to 1 mM. Imidazolium imidazoleoxo-bisperoxovanadate(V), was also effective *in vitro* (1 μM V) at increasing insulin receptor phosphorylation up to 4 times greater than maximal insulin stimulation, and in increasing glucose uptake substantially (in rat adipocytes and epitrochlearis muscle) in the presence of submaximal insulin [106].

Peroxovanadates (pV) in general are more efficient than vanadate at oxidative coupling of bio-molecules such as cysteine and citrate [107,108]. Unfortunately, they are not orally available and are not terribly stable [109]. In addition, abnormalities of lactate metabolism have been a recurring negative side effect with attempted *in vivo* use of peroxovanadates as antidiabetic drug candidates [5,110].

Since hydroxylamine is isoelectronic with hydrogen peroxide, vanadium(V) complexes of hydroxylamine analogues [111,112] might be

expected to exhibit some structural similarities to peroxovanadates. Nonetheless, hydroxylamine vanadyl complexes appear to be much weaker oxidants of thiol groups than are peroxovanadates and, although they are also potent inhibitors of some PTPases, the mechanisms for the two types of complexes appear to differ.

3.6. Vanadium Complexes with Synergistic Potential

Chelation is an excellent way of fine-tuning the properties of a metal ion, but it is also possible to introduce substituents that incorporate known pharmaceutical agents for synergistic potential. Because it appears that vanadium insulin enhancing compounds lose their structural integrity shortly after administration and absorption (*vide infra*), we have also pursued a synthetic strategy in which the ligand itself has a potential for synergistic action [113,114]. Using this synthetic strategy, several V(IV) complexes have been designed based on known hypoglycemic drugs in use for type 2 diabetes. A series of biguanides, including metformin, were complexed to vanadium to produce vanadyl metformin and analogues [113]. Biguanides are frequently-prescribed oral hypoglycemics for the treatment of T2DM; they decrease insulin resistance through decreased hepatic glucose production and without stimulating insulin secretion [9]. Optimal dosages for metformin are at least one order of magnitude higher than for vanadyl compounds, a fact that mitigated against synergistic activity [9,80,115]. *In vitro* leptin secretion studies comparing metformin and BMOV as glucose uptake stimulators concluded that a linear, insulin-like response could be obtained between 0.1 and 25 mM for metformin, and between 10 and 20 μM for vanadium (for reference, insulin was active at 0.16 nM) [115]. Nonetheless, vanadyl metformin was active when tested in STZ-diabetic rats by oral gavage (though not by i.p. injection), with activity indistinguishable from that of BMOV (and significantly more potent than vanadyl sulfate) [113].

Similarly, thiazolidinedione-containing compounds were used as ligands for $V^{IV}OL_2$ complexes (Figure 3) [114]. A much closer range of therapeutic doses [116,117] suggested that these compounds might be more likely to constitute truly bifunctional antidiabetic compounds [11,118]. A series of vanadium compounds, chelated by ligands containing

a thiazolidinedione moiety were synthesized to create potentially synergistic compounds. The ligand precursors and their complexes were tested for insulin-enhancing potential in STZ-diabetic rats at 0.1 mmol kg^{-1} and compared to rosiglitazone and BMOV, respectively. Both the ligand precursors Hbetd and Habtd showed enhanced activity compared with that of rosiglitazone. The complex $VO(betd)_2$ showed the most efficacious hypoglycemic effects in this study; glucose-lowering in STZ-diabetic rats was comparable to BMOV over the 72 hour testing period, and the effect was more sustained than with the ligand alone [114].

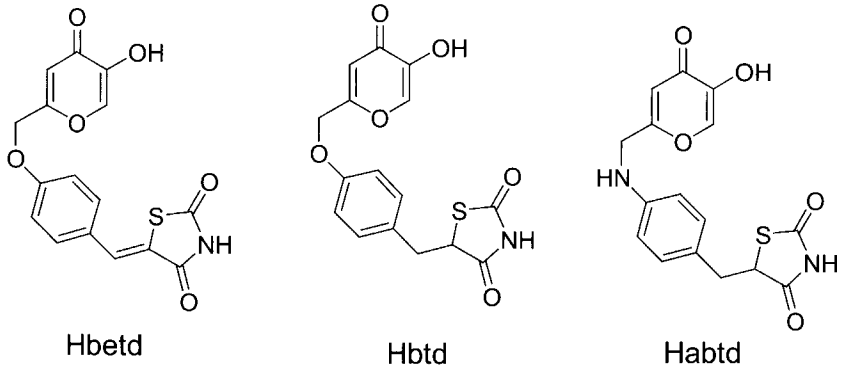


FIG. 3. Thiazolidinedione ligands designed for synthesis of bifunctional vanadium-based insulin enhancing agents.

4. PHARMACOKINETICS AND BIODISTRIBUTION OF VANADIUM-CONTAINING COMPOUNDS

4.1. Distribution in Tissues

No matter how potent a drug candidate may appear in *in vitro* or acute *in vivo* testing, its pharmacological utility is determined by more rigorous and long-term absorption, distribution, metabolism and excretion (ADME) studies and toxicological investigations [119,120]. In this regard, a few selected vanadium compounds have been studied by a variety of techniques designed to reveal *in vivo* biodistribution, following oral, i.p., or s.c. administration.

In multiple studies using ^{48}V-labelled V(V) [121-123], V(IV) [124,125] or chelated V(IV) [125], the order of preferred uptake was found to be similar, with vanadium concentration in bone > kidney > liver ≥ spleen > all other tissues, at 24 hours following oral gavage, i.p. or s.c. administration. Relative organ distribution of vanadium determined by neutron activation analysis (NAA) for VOPIC and close analogues over a longer time period also confirmed this order of tissue uptake [126].

Increased tissue uptake of vanadium from $V^{IV}OL_2$ compared with that from $VOSO_4$, has been demonstrated for BMOV [125,127], VOPIC, VOMPA [126] and bis(5-iodopicolinato)oxovanadium(IV) (VOIPA) [128]. In particular, vanadium in bone was 4-fold greater 24 hours after BMOV than after $VOSO_4$ by oral gavage [125]. Ratios of tissue vanadium concentrations (in STZ-diabetic rats) for bone:liver were 7:1 for BMOV after 25 weeks of oral administration [129]; 11:1 for VOMPA and 35:1 for VOPIC after i.p. administration for 11 days, followed by 7 days of no treatment [126]. Clearly, changes in ligand chemistry can result in major changes in biodistribution and, potentially, in long-term efficacy of the vanadyl complex as an insulin enhancing agent.

4.2. Compartmental Modeling

Compartmental modeling, a method of describing mathematically the uptake, distribution and clearance of biomolecules from the body [130,131], has been used to analyze the radioisotopic decay following administration of ^{48}V-labelled compounds, or the disappearance over time of unlabelled exogenous vanadium [77a,123,125,132-135]. Overall, the evidence suggests that vanadium has a mean residence time (MRT) on the order of several weeks to months in bone following a single oral or i.p. dose of inorganic vanadium salts or chelated vanadium compounds [77a,123,125,132]. By contrast, both a SAAM (simulation and analysis modeling) [131] analysis of radioisotopic decay from ^{48}V-BMOV (0.05 mmol V kg^{-1}), and a blood circulation monitoring-electron spin resonance (BCM-ESR) monitoring of VOPIC and VOMPA (0.01 mmol V kg^{-1}), demonstrated rapid clearance from the bloodstream, with MRT's in systemic circulation of 5-8 minutes [125,133]. Pharmacokinetic models of vanadyl sulfate clearance following i.v.

administration (0.06 mmol V kg^{-1}) [134] predicted a fast phase of disappearance from circulation with a half-life ($t_{1/2}$) of 54 minutes, and of [ethyl-1-^{14}C]BEOV (0.144 mmol V kg^{-1}, by oral gavage) [77a] a $t_{1/2}$ in whole blood of 52 minutes (equivalent to MRT = 75 minutes). Using gelatin or enteric-coated capsules for vanadyl sulfate delivery permitted extension of blood MRT to 5.4 and 11.7 hours, respectively [135].

An important feature of tracking the disappearance of [ethyl-1-^{14}C]BEOV after oral ingestion was the simultaneous determinations of ligand and vanadium ion redistribution *in vivo* (vide infra) [77a]. Curves of disappearance of plasma and whole blood ^{14}C and V diverged dramatically within the first hour after administration of the vanadium complex. In addition, neither the tissue V and ^{14}C concentrations nor any of their pharmacokinetic parameters were in accord for liver, kidney, bone, small intestine or lung, indicating that at least most of the compound was, in fact, dissociated. These results are in agreement with a number of spectroscopic studies, showing rapid dissolution of vanadium complexes in an aqueous environment [84,87,92,136-138].

4.3. Absorption and Bone Uptake

4.3.1. Caco-2 Cells as a Model of Absorption

Given that vanadium compounds appear to disintegrate fairly soon after ingestion, differential effects of the ligand on the insulin enhancing activity may be most closely related to changes in absorption characteristics propagated by the ligand binding. Cellular models of intestinal absorption are therefore being applied to comparisons of tissue uptake and distribution of vanadium compounds [127]. So far, Caco-2 cells, a human carcinoma cell line validated as a model of gastrointestinal absorption [120,139], were used to compare vanadium absorption from BMOV with that from vanadyl sulfate or ammonium metavanadate [127]. Intracellular vanadium concentration measured by graphite furnace atomic absorption spectrophotometry (GFAAS) was significantly greater at 60 minutes in BMOV-treated cells compared to those treated with vanadium salts. Further studies are needed to test the hypothesis that absorption is a crucial property of vanadium-containing

coordination complexes in determining their relative efficacy as insulin enhancing agents.

4.3.2. Inclusion of Vanadium in Bone

Vanadium compounds accumulate preferentially in bone (*vide supra*); this may even constitute a reservoir of vanadium for prolonged glucose-lowering effect at a reduced maintenance dose [140]. Molecular modeling of vanadium speciation in BEOV-treated rat bone with electron spin echo envelope modulation (ESEEM-EPR) suggested interaction of vanadyl ions with the hydroxyapatite portion of bone, fairly near the surface of the bone [138,141]. In support of vanadium supplementation having a positive effect on bone, vanadium(V) pentoxide, 0.15-0.20 mmol kg^{-1} for 3 days was shown to stimulate bone formation in weanling rats [142,143], and sodium orthovanadate, 0.03 mM in drinking solutions for 9 weeks to adult female rats, prevented glucocorticoid-induced osteoporosis [144].

4.4. A Tunable Response?

Bioavailability of vanadium-containing drugs is clearly dependent on more than just how much of the intact drug reaches the systemic circulation. In addition to the evidence presented above, concomitant administration of several amino acid hydroxamates with either V(V) or V(IV) salts has been shown to potentiate the glucose-lowering activity of vanadium in STZ-diabetic rats, with only circumstantial evidence that a chelate is formed *in vivo* at some stage in the metabolism of each [145]. Peroxovanadium compounds delivered through the skin (e.g., as a patch) have also been shown to be effective in normalizing blood glucose in STZ-diabetic rats [146]. Transdermal delivery of a test compound, bpV(phen), increased blood levels of vanadium far more effectively than oral administration of the same compound (the latter also had no effect on blood glucose levels). As well, rapid clearance of chelated vanadium compounds is followed by differential uptake into tissues and varying rates of disappearance after single oral or i.p. doses (*vide supra*).

This is not to say that the particular ligand is irrelevant. In fact, the particular ligand influences redox, thermodynamic and aqueous stabilities;

the extent of binding to both small (e.g., ascorbate, citrate, glutathione) [136] and large (e.g., transferrin, serum albumin, metallothionein, calmodulin) biomolecules [87,141]; and the dynamics of uptake and distribution in the body. The particular ligand also, of course, affects aqueous solubility and nonaqueous (i.e., lipid) compatibility.

The range of glucose-lowering efficacies observed in a standardized *in vivo* screen (Table 1) attests to the fact that this is a tunable response. Recent findings with bifunctional vanadium-containing pharmaceutical candidates may point the way to future successes in this area. By taking advantage of the pharmacological relevance of the ligand itself, investigators will be able increasingly to complement vanadium's insulin enhancing effects, leading to a more consistent response at a lower overall effective dose.

5. CONCLUSIONS

Vanadium-based candidate pharaceutical agents can enhance insulin action without increasing plasma insulin levels when administered orally, or in a variety of parenteral modalities. Chelation of vanadium ions with any of a number of organic ligands has proven effective in reducing the therapeutic dose required for glucose- and lipid-lowering effects, and provides an avenue for increased bioavailability with concomitant decrease in likelihood of vanadium overload. Future work will target the design of appropriate dosing regimens for use of vanadium compounds as an adjunct to diabetes therapy.

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ABBREVIATIONS AND DEFINITIONS

%PG	% plasma glucose-lowering $\%PG = (PG_{control} - PG_{test})/PG_{control} \times 100\%$
AGI	α-glucosidase inhibitors
BCM-ESR	blood circulation modeling – electron spin resonance
BEOV	bis(ethylmaltolato)oxovanadium(IV)
BMOV	bis(maltolato)oxovanadium(IV)
bpV(Me_2phen)	potassium oxodiperoxo(1,10-methylphenanthroline)-vanadate(V)
Caco-2	human carcinoma cell model of intestinal absorption
CytPTK	cytosolic protein tyrosine kinase
DCCT	Diabetes Control and Complications Trial
ED_{50}	effective dose (dose at which 50% of test animals respond)
ESEEM	electron spin echo envelope modulation
F26	nontransformed human fibroblast cell line
FFA	free fatty acid
FPG	fasting plasma glucose
GFAAS	graphite furnace atomic absorption spectrophotometry
GI	gastrointestinal
Habtd	5-{4-[(5-hydroxy-4-oxo-4H-pyran-2ylmethyl)-amino]benzyl}thiazolidine-2,4-dione
Hama	allomaltol
HbA_{1c}	hemoglobin A_{1c}
Hbetd	5-[4-(5-hydroxy-4-oxo-4H-pyran-2ylmethoxy)-benzylidene]thiazolidine-2,4-dione
Hbtd	5-[4-(5-hydroxy-4-oxo-4H-pyran-2ylmethoxy)-benzyl]thiazolidine-2,4-dione
Hdtpp	1,2-dimethyl-3-hydroxy-4-thiopyridinone
Hema	2-ethyl-3-hydroxy-4-pyrone
Hepp	1,2-diethyl-3-hydroxy-4-pyridinone
Hetma	2-ethyl-3-hydroxy-4-thiopyrone

Hima	isomaltol
Hipma	3-hydroxy-2-isopropyl-4-pyrone
Hma	3-hydroxy-2-methyl-4-pyrone
Hmpp	3-hydroxy-2-methyl-4-pyridinone
Hmtpp	3-hydroxy-2-methyl-4-thiopyridinone
Hnbma	2-*n*-butyl-3-hydroxy-4-pyrone
Htma	3-hydroxy-2-methyl-4-thiopyrone
i.p.	intraperitoneal
LDL	low density lipoprotein
LED	lowest effective dose
mpV(2,6-pdc)	pyridine-2,6-dicarboxylatooxoperoxovanadate
mpV(pic)	picolinatooxoperoxovanadate
MRT	mean residence time ($MRT = t_{1/2} \div 0.693$)
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide
NAA	neutron activation analysis
NIDDM	non-insulin dependent diabetes mellitus
pbV(phen)	potassium oxodiperoxo(1,10-phenanthroline)-vanadate(V) trihydrate
PTPase	protein tyrosine phosphatase
pV	peroxovanadate
s.c.	subcutaneous
SAAM	Simulation, Analysis and Modeling software
STZ	streptozotocin
SV 3T3	Simian virus transformed Swiss 3T3 mouse fibroblasts
T2DM	type 2 diabetes mellitus
$VO(acac)_2$	vanadyl acetoacetonate
VOIPA	vanadyl iodopicolinic acid
VOMPA	vanadyl methylpicolinic acid
VOPIC	vanadyl picolinate
$VOSO_4$	vanadyl sulfate
ZDF	Zucker diabetic fatty

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