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Vanadium and diabetes

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Abstract

We demonstrated in 1985 that vanadium administered in the drinking water to streptozotocin (STZ) diabetic rats restored elevated blood glucose to normal. Subsequent studies have shown that vanadyl sulfate can lower elevated blood glucose, cholesterol and triglycerides in a variety of diabetic models including the STZ diabetic rat, the Zucker fatty rat and the Zucker diabetic fatty rat. Long-term studies of up to one year did not show toxicity in control or STZ rats administered vanadyl sulfate in doses that lowered elevated blood glucose. In the BB diabetic rat, a model of insulin-dependent diabetes, vanadyl sulfate lowered the insulin requirement by up to 75%. Vanadyl sulfate is effective orally when administered by either single dose or chronic doses. It is also effective by the intraperitoneal route. We have also been able to demonstrate marked long-term effects of vanadyl sulfate in diabetic animals following treatment and withdrawal of vanadyl sulfate. Because vanadyl sulfate is not well absorbed we have synthesized and tested a number of organic vanadium compounds. One of these, bismaltolato-oxovanadium IV (BMOV), has shown promise as a therapeutic agent. BMOV is 2–3× more potent than vanadyl sulfate and has shown less toxicity. Recent studies from our laboratory have shown that the effects of vanadium are not due to a decrease in food intake and that while vanadium is deposited in bone it does not appear to affect bone strength or architecture. The mechanism of action of vanadium is currently under investigation. Several studies indicate that vanadium is a phosphatase inhibitor and that vanadium can activate serine/threonine kinases distal to the insulin receptor presumably by preventing dephosphorylation due to inhibition of phosphatases Short-term clinical trials using inorganic vanadium compounds in diabetic patients have been promising. (Mol Cell Biochem **188**: 73–80, 1998)

Key words: vanadium, diabetes, glucose lowering, insulin-mimetic

Introduction

As we approach the 21st century, diabetes research has never been so intense. Along with important improvements in the scientific tools and techniques, deep insights into the complex inter-relationship between insulin action, insulin resistance, lipid and carbohydrate metabolism have been made. Twelve years ago, a very surprising discovery was made *in vivo* demonstrating that vanadium can enhance and/or mimic the physiological effects of insulin in an experimental model of diabetes [1]. Since this first observation, a great deal of work has been done and recently data demonstrating beneficial effects of vanadium in humans have been published leading to the question, 'could vanadium be a useful adjunct in the treatment of diabetes?'. Key aspects of the findings with this unique trace element in relation to its insulin-mimetic, antihyperglycemic and antihypertensive effects are reviewed in the following pages.

Background

Vanadium was discovered in 1813 by the mineralogist Del Rio who gave it the name *panchromium* because of its color changes as a function of its oxidation state. This transitional element was then rediscovered in 1831 by the Swedish chemist Nils Gabriel Sefstom who named the compound *vanadis*, a nickname of the Germanic goddess of beauty. In

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common with most transitional metals, vanadium exists in several valence states (–3, –1, 0, +1 to +5) and the expression of a given form is highly pH dependent. In biological systems vanadium is found predominantly as the vanadate (+5) and vanadyl (+4) forms. In plasma, vanadium exists in both oxidation states. Approximately 90% is bound to proteins (predominantly transferrin)[2]. Most ingested vanadium is transformed in the stomach to V02+ and remains in this form as it passes through the duodenum. Vanadium has been shown to be stored in the bone (main storage depot), kidney and liver following i.p. injection [3]. In humans, the total body pool of vanadium is estimated to be 100–200 µg [4].

In-vitro insulin mimetic actions of vanadium

In vitro, the insulin-like effects of vanadium extend to numerous processes involved in carbohydrate, lipid and protein metabolism: (i) carbohydrate metabolism through glucose transport, glucose transport translocation, glycolysis and glycogen synthesis [5–11]; (ii) lipid metabolism primarily by inhibition of lipolysis, and (iii) protein metabolism and mitogenesis [12–16].

In-vivo insulin mimetic actions of vanadium

The first report of vanadium's insulin mimetic and antidiabetic potential *in vivo* was published by Heyliger *et al*. [1]. This study was performed on streptozotocin (STZ) diabetic rats chronically treated with sodium orthovanadate. Normalization of hyperglycemia and improvement of cardiac depressed function were both recorded without an increase in plasma insulin levels in the diabetic rats. This observation demonstrated the ability of vanadium to improve insulin sensitivity. Hyperinsulinemic clamp studies later confirmed a decreased insulin resistance following vanadium treatment [17]. In 1987, Meyerovitch showed that in addition to lowering plasma glucose levels, chronic sodium metavanadate administration could also enhance basal hexose transport in both liver and muscle [18]. Brichard *et al*. subsequently described a dose-response relationship between vanadate and its glucose lowering effects [19]. A summary of the most prominent effects of vanadium in STZ diabetes is shown in Table 1.

Since vanadyl sulfate was reported to be 6–10 times less toxic than vanadate [20] this form of vanadium was extensively investigated for its insulin-like effects. STZ-diabetic animals responded to vanadyl sulfate given in drinking water with

Table 1. In vivo effects of vanadium in STZ-diabetes

· Amelioration of insulin resistance reflected by a greater glucose lowering effect of vanadium treated rats to insulin [25].
· Normalization of both basal and stimulated hepatic glucose production by chronic vanadium administration [59].
· Enhanced insulin sensitivity in vanadium treated rats correlates with restoration of insulin stimulated MAP and S6 kinase activities in skeletal muscle [47].
· Chronic vanadium treatment corrects abnormalities in glycolytic enzymes i.e. phosphofructokinase-2 and glucokinase [60].
· Restoration of glycogen synthase and phosphorylase activies [61].
· Aberrations in the tissue specific expression of 2 isoforms of glucose transporter in STZ-diabetes are normalized by vanadium treatment [62].
· Amelioration of oxidative stress [63].
· Long term effects on glucose metabolism following oral treatment and withdrawal [24].
· Prevention of secondary cardiac complications such as cardiomyopathy and cataracts [1, 23, 27].
· Prevents long-term secondary complications
· Decreases elevated triglyceride and cholesterol levels [23, 27]
· Prevents plasma elevations of urea and creatinine [29]
· Restores thyroid hormone levels to normal [23, 27]

corrected plasma glucose, lipids, creatinine and thyroid hormone blood levels. These improvement were accompanied by correction of abnormalities in isolated heart function and glycerol output from adipose tissue [22].

Ramanadham *et al*. in 1989 [23] published a very interesting result involving vanadyl sulfate. In the study, STZ diabetic animals were orally treated for 3 weeks with vanadyl sulfate and then the treatment was withdrawn for 13 weeks. At the end of this period the authors recorded a sustained prevention of myocardial and metabolic aberrations, along with normal blood levels of glucose, insulin, lipid and thyroid hormones. In addition, corrected glycerol output from adipose tissue and no evidence of cataract development could be observed. Together, these findings strongly suggested that vanadium could produce a sustained insulin-like effect on these animals despite the fact that vanadium was no longer present in the body. These animals were not completely normal in that a challenge with glucose did not produce further insulin release and resulted in an abnormal glucose tolerance curve.

In order to better understand the basis of the antidiabetic effects of vanadyl sulfate, Cam *et al*. conducted an experiment to test the hypothesis of a putative prophylactic action of vanadium against the cytotoxic destruction of the pancreatic beta cells by STZ [24]. Diabetes was induced by STZ injection and vanadyl sulfate treatment started 3, 10 or 17 days later and lasting for a 5 month period. Irrespective of the delay separating diabetes induction and beginning of the treatment, parameters such as glucose tolerance and adipose tissue function were normalized in diabetic-treated animals.

These observations are therefore not in favor of the concept that vanadium efficacy as an insulin mimetic is due to a protective effect of the endocrine beta cells from the deleterious effects of STZ.

Concentration dependent effects and the *in vivo* interaction of vanadyl with insulin was studied by Battell *et al*. [21] and Ramanadham *et al*. [25]. Using the diabetic BB rat, a spontaneous Type I model of diabetes which produces no insulin, vanadyl sulfate produced dose-dependent effects which reduced the exogenous insulin requirement necessary to prevent glycosuria by 75%. Two important findings were obtained from this set of results: (1) vanadium requires the presence of some insulin to produce its *in vivo* effects, therefore, (2) vanadium *in vivo* is an insulin enhancer [21, 25].

Vanadium effects have also been demonstrated in other models of both Type I and Type II diabetes and these are summarized in Table 2.

The use of organic vanadium complexes to increase potency

In order to overcome poor absorption of vanadate and vanadyl from the gastrointestinal (GI) tract and GI toxicity, i.e. diarrhea, our laboratory and others have synthesized various organic vanadium compounds which were designed to improve absorption, potency and therapeutic safety.

Bis(maltolato)oxovanadium (IV) (BMOV), a maltol/vanadyl compound, was developed in collaboration with Dr. C. Orvig in the Department of Chemistry at the University of British Columbia, Vancouver, Canada [26]. BMOV is an example of a series of compounds specifically designed to be administered orally and absorbed by passive diffusion. It is water soluble, electrically neutral and has a low molecular weight [26, 27]. BMOV and vanadyl sulfate have been compared for their effects by both oral administration and intraperitoneal (i.p.) administration using a single dose [28]. The ED_{50} following oral administration indicated that the ED_{50} for BMOV (0.5 mmol/kg) was twice as potent as vanadyl sulfate (ED_{50} - 0.92 mmol/kg). Following i.p. administration, BMOV was 3 times more potent than vanadyl sulfate. In addition, it was interesting to note that the highest BMOV dose produced euglycemia in 100% of the treated animals whereas vanadyl sulfate produced the effect in only 80–90% of the animals.

Chronic BMOV treatment (0.75 mg /ml in drinking water) over a period of 6 months in STZ-diabetic rats restored plasma glucose levels to normal (8/12 animals) as well as heart function in all diabetic-treated rats. However, BMOV did not affect body weight gain in control rats for the first 10 weeks when compared to vanadyl sulfate [28]. This last observation is important regarding some concerns expressed in the literature about vanadium toxicity. Dai *et al*. in our laboratory evaluated the effects of long-term BMOV and vanadyl sulfate treatments on several pathological determinants of STZ-induced diabetes [29]. Chronic BMOV treatment completely prevented elevations in plasma urea, creatinine, alanine aminotransferase (ALT) and improved histological abnormalities in the kidney and liver from STZ-diabetic rats.

BMOV was also used to assess organic vanadium effectiveness in a Type II model of diabetes, the fa/fa Zucker rat. BMOV at a maximal concentration of 0.5 mg/ml for 14 weeks of treatment did not affect body weight gain in lean controls but did significantly reduce body weight gain in the fatty-treated group. At this concentration, BMOV also significantly reduced plasma insulin levels. A lower concentration of 0.2 mg/ml BMOV did not affect food or fluid intake and did not decrease body weight or plasma cholesterol levels in fatty-treated animals. The lower dose also significantly reduced plasma, insulin and triglyceride levels. Oral glucose tolerance was also improved by BMOV treatment in the fatty animals even at this lower dose [30].

Table 2. In vivo effects of vanadium in other models of diabetes

- Spontaneouslv Diabetic BB rat
  Reduces the dose of insulin required by 75% [21, 25]
- Partiallv Pancreatectomized Rat
  Improves insulin-sensitivity towards peripheral glucose uptake in 90% pancreatectomized rats predominantly through correction of glycogen synthesis [64]
- Neonatal STZ-Diabetic Rats
  Vanadium treatment corrects basal and stimulated hepatic and glucose production and peripheral glucose utilization [65]
- Genetically obese fa/fa (Zucker) Rats
  Attenuates hyperinsulinemia and impaired glucose tolerance [30, 66]
  Lowers blood lipids, dose dependent decrease in weight gain [30]
- Obese ob/ob Mice
  Attenuates hyperglycemia, improves glucose tolerance and hepatic glycogen content. Prevents pancreatic exhaustion of insulin [67]
- Zucker Diabetic Fatty Rats
  Attenuates hyperglycemia, hyperinsulinemia and hyperlipidemia. Restores glucose tolerance and decreases pancreatic insulin depletion (Yuen and McNeill, unpublished observations).

Vanadium compounds ameliorate insulin resistance hyperinsulinemia and hypertension

Extensive epidemiological, clinical and experimental data lend credence to the association between essential hypertension and abnormalities in carbohydrate and lipid metabolism [31–33]. Hyperinsulinemia and insulin resistance are glucose

metabolism related defects frequently found associated with hypertension. They are also linked to a high atherogenic risk profile, dyslipidemia and atherosclerosis. It would then be expected that drugs interventions that corrected these defects would also decrease blood pressure if there is a correlation between the events. In order to test the hypothesis of a potential relationship between hyperinsulinemia, insulin resistance and hypertension, we undertook a series of experiments, using vanadyl sulfate and BMOV. The study was conducted in both a genetic and an acquired model of hypertension, respectively: the spontaneously hypertensive rat (SHR rat) and the fructose hypertensive rat. Vanadium compounds reduced plasma insulin levels and blood pressure in both types of animals. Moreover, administration of exogenous insulin to match the level of the untreated animals reversed the beneficial effects of vanadium on blood pressure. These results strengthen the hypothesis of the link between hyperinsulinemia/insulin resistance and hypertension and demonstrate the antihypertensive potential of vanadium *in vivo* [17, 34–36].

Mechanism of action

The mechanism of action of vanadium in producing its antidiabetic effects *in vivo* is poorly understood and is currently the subject of much investigation. *In vitro* and *in vivo* data demonstrate that vanadium does affect various aspects of the insulin signaling pathway (Table 3).

It was postulated that vanadium's insulin mimetic effects would be the result of vanadium behaving as a phosphate analog and stimulating protein tyrosine phosphorylation through inhibition of protein tyrosine phosphatases (PTPases) [37, 38]. Thus, it was not surprising to record vanadium induction of autophosphorylation of solubilized insulin receptor (IR) in a fashion analogous to insulin [39, 40] with stimulation of the tyrosine kinase activity of the IR beta subunit [41]. Other studies however demonstrated that vanadium was equally effective in stimulating glucose metabolism in rat fat cells when half the IR had been inactivated by insulin over stimulation [42]. Furthermore, glucose-lowering effects with oral vanadium treatment were observed while no IR kinase activity change could be recorded [43]. These last two observations and others [44, 45] are in favor of potential post-receptor effects of vanadium, further downstream in the insulin signaling cascade. Knowing that intracellular vanadium is primarily in the vanadyl form (which is not a potent phosphatase inhibitor), it is reasonable to speculate that other signaling molecules in the insulin signaling pathways might be targets for vanadium action. In addition, the effects of vanadium on intracellular calcium flux, intracellular and intravesicular pH should not be underestimated in studying the insulin-mimetic effects of vanadium.

Table 3. Suggested effects of vanadium on insulin-signaling pathways *in vitro*

- Stimulates autophosphorylation of insulin receptors [39, 40]
- Increases insulin receptor tyrosine kinase activity [41]
- Stimulates down regulation of insulin receptors [46]
- Increases insulin receptor binding [46]
- Increases protein tyrosine kinase activity [44]
- Increases Ser/Thr protein kinase activity [47, 48]
- Inhibits phosphotyrosine phosphatase activity [37, 38]

A study was conducted in intact rat adipocytes where vanadium was shown to activate a staurosporine sensitive cytosolic protein tyrosine kinase (Cyt PTK) distinct from IR tyrosine kinase. This activation was linked to glucose oxidation and lipid synthesis but dissociated from glucose uptake and inhibition of lipolysis. Cyt PTK would then be implicated only in specific cellular response. Furthermore, Cyt PTK would be highly selective for vanadium since neither insulin, isoproterenol, dibutyryl cAMP, okadaic acid, hydrogen peroxide or phorbol ester TPA did affect Cyt PTK activity. In addition to vanadium, other PTPase inhibitors have also been shown to activate Cyt PTK in adipocytes. It should be noted that insulin mimetic effects of vanadium on hexose uptake and inhibition of lipolysis are not blocked by staurosporine (a blocker of Cyt PTK) indicating that this pathway is dehmitely not the only means by which vanadium influences cellular physiology [46].

Insulin signal transduction is mediated intracellularly through a complex network of cascades of reversible protein phosphorylations and dephosphorylations. Among the numerous kineses involved in insulin signaling, MAP and S6 kinases have been demonstrated to be defective in both the basal and insulin stimulated state in STZ-diabetic rats. We found that chronic vanadium treatment corrected the insulin-induced activation of these kinases [47, 48]. Thus, insulin resistance associated with long-term diabetes may be linked with altered signaling through these kinases and vanadium could rectify the observed defects. Preliminary results from recent experiments in our laboratory indicate that the picture may be more complex regarding the relative importance of different kinases as potential vanadium targets in order to explain its insulin mimetic effects and its ability to correct insulin resistance.

The long-term effects of vanadium treatment were further investigated in our laboratory. Since a persistent euglycemic state can be observed following vanadium treatment withdrawal with only minor improvements in pancreatic secretory function, several hypothesis have been proposed. The vanadium-treated rats could sustain an increased sensitivity to circulating insulin even after the treatment has been stopped. A second possibility could be that vanadium is

released from potential tissue storage sites producing the anti-hyperglycemic effects although this seems highly unlikely. Alternatively, Cam *et al*. [49] suggest that vanadium-induced amelioration of the diabetic state may be partially due to preservation of a functional portion of pancreatic beta cells in the STZ animals. This study showed that a modest increase in β cell content was crucial to the long-term effect of vanadium even though the total insulin content was still much less than normal. It remains clear that the absence of normal plasma insulin levels strongly suggests the presence of additional actions of vanadium, perhaps at the level of insulin sensitive tissues [49].

Clinical studies

Some very elegant studies have recently been conducted on both Type I and Type II human diabetic subjects. Sodium metavanadate was administered for 2 weeks at 125 mg daily in divided doses. In Type I diabetic patients, vanadium lowered insulin requirements without an effect on C-peptide levels suggesting the absence of an influence on insulin release. In addition, 2 out of the 5 insulin-dependent subjects showed improved glucose utilization. In Type II diabetic patients, improved insulin sensitivity, enhancement of non-oxidative glucose disposal rates and higher basal MAP and S6 kinases activity in monocytes were recorded. Hepatic glucose production was not affected. Diarrhea was the main side effect observed [50].

Vanadyl sulfate was also studied in 6 non-insulin-dependent diabetic patients at a dose of 100 mg/day for 3 weeks. Reduced fasting plasma glucose and HbAlc were recorded without an effect on plasma insulin levels. An interesting finding was the persistence of improved insulin sensitivity for up to 2 weeks following cessation of the treatment [51]. This last observation agrees with experimental studies described earlier. Additional recent reports tend to validate the observation of beneficial effects of vanadyl and vanadate treatment in non-insulin-dependent human subjects [52, 53].

Vanadium glucose lowering effects and food restriction

Vanadium compounds have now been extensively demonstrated to normalize the hyperphagia associated with experimental diabetes. In 1994, Malabu *et al*. stated some concerns claiming that the decrease in plasma glucose levels observed after vanadate administration was entirely attributable to a reduction in food intake [54].

In our laboratory, Yuen *et al*. conducted a detailed study on STZ-diabetic rats to precisely define the respective effects attributable to vanadium alone or to food restriction alone. Two main parameters were used to assess these influences: plasma glucose levels and lipid levels [55]. STZ-diabetic rats were treated daily over a 6 week period with BMOV dissolved in drinking water. Pair-fed groups were fed based on the intake of their respective counterparts from the previous day. Decreases in plasma glucose, triglycerides and cholesterol levels in diabetic-treated rats were recorded with no effect on the plasma insulin level. None of these parameters were affected in pair-fed animals. In addition, prevention of cardiac function impairment was observed in STZ-diabetic rats treated with BMOV but not in pair-fed diabetic animals. The experimental design used in Malabu's study may explain the different results since food was given to pair-fed animals only once a day. Our observation is that hyperphagic diabetic animals consume this small amount of food in a very short period of time after provided. The animals are therefore left fasting for a long period of time since blood was not collected until the morning. This factor is crucial since reduction in plasma glucose levels in their study for pair-fed diabetic groups was similar to what we have observed after a prolonged (20 h) period of fasting.

Vanadium in bone

Concern has been raised by the fact that vanadium deposits in bone and could be potentially toxic [56]. Indeed studies have shown that chronic vanadium administration to rats results in bone concentrations of vanadium of 10–26 μg/g [57, 58]. These concentrations were twice that of the accumulation in kidney and 6–10 times higher than those found in liver [57, 58]. In order to test the effects of vanadium on bone strength, we examined the tibia and vertebrae from Zucker Diabetic Fatty rats treated with BMOV at concentrations in the drinking water beginning with 0.2 mg/ml increasing incrementally to 0.8 mg/ml over a 10 week period. The treatment with BMOV improved blood glucose in the animals from 28.7 mM to 13.3 mM and modestly improved plasma triglyceride levels. Polydypsia was improved and there was a slight (8%) decrease in body weight. Vanadium content of treated rats was 9.42 ± 1.72 (S.D.) μg/g for tibia and 6.60 ± 1.10 μg/g for vertebrae, the difference is likely due to the fact that vertebrae have a lower content of bone than tibia. This is evident if we consider the vanadium/phosphate ratio (0.79 ± 0.15 for tibia, 1.07 ± 0.19 for vertebrae) is higher for vertebrae thus reflecting the higher turnover of trabecular bone (vertebrae). Vanadium treatment did not affect the content of other minerals in the bone (K, Mg, Na, Ca, P) nor did it affect bone crystal size as determined by X-ray diffraction. The mechanical properties of the tibia as reflected by the 3-point bending test and of vertebrae as determined by compression testing were not affected by

vanadium administration. Finally image analysis of a stained thin section of vertebrae showed no changes in bone architecture in vertebrae of rats treated with BMOV. Thus the architecture, density and mechanical properties of bone were not affected by the treatment with vanadium in doses which produced insulin-like effects. Further studies over longer periods of time will be required to fully determine the effects of vanadium on bone.

Conclusion

Since our initial demonstration of the anti-diabetic effects of vanadium *in vivo*, significant advances have been made in understanding the glucose-lowering properties and the mechanism(s) of action of vanadium compounds. The exact intracellular mechanism and/or mediators involved in vanadium actions remain unknown but vanadium effects may be mediated by a synergy between several post-receptor events of the insulin signaling cascade in the target tissues of the hormone. Design and development of organic ligands with improved absorption, tissue uptake, potency, and having decreased toxicity is important. BMOV exemplifies one such organically chelated complex that appears to be a potent insulin-mimetic and insulin enhancer. BMOV demonstrates less gastrointestinal side effects and does not affect body weight gain and food and fluid intake in control-treated rats.

Vanadium research demonstrating the antihypertensive effects of vanadium compounds in hyperinsulinemic and insulin-resistant models of hypertension may also prove to be important. Early trials with vanadium in diabetic human volunteers have shown promising results consistent with experimental studies. Within the next few years the possible therapeutic roles of vanadium should be more clearly established.

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