Potential Use of Vanadium Compounds in Therapeutics

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Abstract: Vanadium is a trace element present in practically all cells in plants and animals. While the essentiality of vanadium for human beings remains to be well established, vanadium has become an increasingly important environmental metal. Vanadium compounds exert a variety of biological activities and responses. At pharmacological doses, vanadium compounds display relevant biological actions such as insulin and growth factor mimetic or enhancing effects, as well as osteogenic and cardioprotective activity. On the other hand, depending on the nature of compounds and their concentrations, toxicological actions and adverse side effects may also be shown. Nevertheless, the toxic effects may be useful to develop new antitumoral drugs. In this review, the authors summarize current knowledge and new advances on *in vitro* and *in vivo* effects of inorganic and organically-chelated vanadium compounds. The effects of vanadium derivatives on some cellular signaling pathways related to different diseases are compiled. In particular, the pathways relevant to the insulin mimetic, osteogenic, cadioprotective and antitumoral actions of vanadium compounds have been comprehensively reviewed. The knowledge of these intracellular signaling pathways may facilitate the rational design of new vanadium compounds with promising therapeutic applications as well as the understanding of secondary side effects derived from the use of vanadium as a therapeutic agent.

Keywords: Vanadium compounds, insuinmimics, antitumoral, osteogenic, cardioprotection, intracellular pathways, ERK and PI3-K pathways, oxidative stress.

INTRODUCTION

Vanadium is an ultra trace element present in higher plants and animals [1,2]. The essentiality, biodistribution, and toxicology of vanadium, like its biological and pharmacological activity are areas of increasing research and widespread interest. It has been demonstrated that vanadium compounds exhibit interesting biological and pharmacological properties. In particular, vanadate(V) and vanadyl(IV) derivatives show insulin-mimetic/ antidiabetic activity**,** growth factor and osteogenic actions [3-5], antitumoral properties [6,7], cardioprotective actions [8, 9] and neurologic effects [10]. Foods are the major source of vanadium for the general population, with a range of 0.1-10μg/kg of wet tissue. Vanadium content in food varies greatly among different types of food. Although most of foods contains a low amount of vanadium (<1 ng/g) [11], some others like mushrooms, shellfish, dill seed, parsley and black pepper are rich in vanadium [12]. In mammalian tissues, vanadium concentration ranges between 0.014-7.2 μM with an estimated amount of 100 – 200 μg in humans [13, 14]. While vanadium essentiality for some lower organisms has been demonstrated, convincing evidence to support an essential role for this element in humans is still lacking [15]. The estimated daily "dietary" intake of vanadium in US population ranges from 10 – 60 μg vanadium. Once absorbed, vanadium distributes among different tissues associated with cytoplasm proteins and in the blood cells, vanadium(V) is reduced to vanadium(IV) by cytoplasm glutathione [16]. Then, vanadium is mainly stored in bone [1]. The ability of vanadium to regulate multiple intracellular signalling pathways has suggested its use as a possible therapeutic tool for the treatments of different diseases. This review addresses the extent to which the known actions of vanadium on cell regulatory processes can account for its insulinmimetic, osteogenic, antitumor and cardioprotective actions.

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INSULIN MIMETIC ACTIONS OF VANADIUM COMPOUNDS

Towards the end of 19th century, the first treatments of diabetic patients by oral administration of inorganic vanadium(V) salts were carried out in France. Prescriptions were based on sodium orthovanadate and metavanadate. Two of the three treated patients showed a decrease in their glycosuria but, on the other hand, some toxic side effects could also be observed during these treatments [17].

Studies performed over the last three decades have established the ability of vanadium compounds either as vanadium(V) and (IV) inorganic species or as organic chelate derivatives to exert different insulin-mimetic and antidiabetic effects *in vitro* and *in vivo* (reviewed in [1, 18,19]). The first reports of insulin-mimetic actions of vanadium were done by Tolman and Partridge [20] and by Shechter and Karlish [21]. These studies were carried out *in vitro* using the inorganic forms of vanadium(V) and (IV), respectively. McNeill and co-workers reported the first evidence of anti-diabetic actions of vanadate(V) *in vivo* [22]. The effect of vanadate on blood glucose was assessed in female Wistar rats treated with streptozotocin (STZ). When vanadate (0.8 mg/ml in drinking water) was administered to the diabetic rats for a 4-week period, their blood glucose was not significantly different from that of nondiabetic rats [22]. In the same way, Shechter and co-workers showed that oral administration of vanadate (0.8 mg/ml) normalized blood glucose levels in STZ-treated rats within 2-4 days of application and led to the appearance of hypoglycemia in the test animals. Lower concentrations of vanadate (0.2 mg/ml in drinking water) also lowered blood glucose levels within 4 days, but did not lead to hypoglycemia for at least 3 weeks. This last treatment caused a stable anabolic and normoglycemic state in STZ-rats [23]. Intracellular vanadium may fluctuate between vanadate(V) and vanadyl(IV) according to the prevailing conditions [24-26]. An interesting discovery was realized by Sakurai who detected vanadyl(IV) in subcellular fractions of liver by ESR spectroscopy after i.p. injection of

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pentavalent vanadium(V) as sodium vanadate into rats for three days [27]. Since vanadyl(IV) cation is less toxic to living organisms and cells than vanadate(V) [28], different studies were performed with vanadyl(IV) cation and derived compounds. Vanadyl sulphate showed anti-diabetic actions in diabetic animals, despite having lower body weights and insulin levels; the animals showed normal plasma concentrations of glucose, lipid, creatinine, and thyroid hormone [29].

Using another model that resembles type 2 diabetes, Brichard and co-workers [30] showed the effectiveness of vanadium(V) to ameliorate the symptoms of this type of diabetes. Oral vanadate produced a sustained improvement of glucose homeostasis in genetically insulin-resistant rats (Zucker fa/fa rats). Another study showed that vanadate could improve glucose homeostasis in genetically obese hyperglycemic insulin-resistant ob/ob mice, which presented metabolic abnormalities similar to those of human non-insulin-dependent diabetes [31]. Similar results were obtained by Meyerovitch and co-workers [33] for vanadate in hyperinsulinemic rodent models of non-insulin-dependent diabetes mellitus (NIDDM).

Subsequently, the development of new complexes of vanadium(V) and (IV) with different organic ligands has been an important advance in the potential use of vanadium compounds with pharmacologic properties. The principal aim of this development was to improve substantially the absorption, tissue uptake and intracellular behavior of vanadium compounds, thereby reducing the dose required for optimal effects [33]. Desirable characteristics for these series of vanadium compounds include partial stability in solutions, neutral charge, high lipid solubility, to present potent phosphatase inhibitory action, and finally, sustained, more potent and effective pharmacological effects [34]. For these purposes, different vanadium complexes were synthesized and assessed as potential insulin-mimetic compounds [33-38]. There is one exception to the above, L-Glu(γ)HXM is a vanadium chelator, which unlike the others, penetrates into cells *via* the glutamine transporter, and turns a 'dormant' intracellular vanadium pool into an insulinomimetic-active species [39].

In the last five years, other new classes of vanadium complexes were synthesized using different ligands and strategies. Saccharides [40], polyalcohols [41], oxodiacetic acid [42], hydrazide [43], arylalkylamine [44], 4-amino-2,6-dipicolinate [45], chloro-substituted dipicolinate [46], 3-hydroxy-4-pyridinonate [47], pyridinone and salicylaldehyde [48], salicylaldehyde semicarbazone [49], and nitrilotriacetate anion [50] among others [51, 52] were used as ligands. The main complexes studied *in vivo* both in animal models and diabetic patients were bis(maltolato)oxovanadium(IV) (BMOV) and bis(ethylmaltolato) oxovanadium(IV) (BEOV). The first of these vanadium compounds normalized blood glucose, lipid metabolism, restored food and fluid intake to control levels and improved cardiac dysfunction in STZ-induced diabetic rats without any increase in the circulating insulin levels. The potency of BMOV was about 50% greater than that of vanadyl sulfate and a reduction in the time of action was observed. In addition, toxicity, notably dehydration due to failure to drink, which sometimes has been seen with vanadyl sulfate, was not observed with BMOV [33, 52, 53]. Since 1995, some *in vivo* studies were performed in human patients to determine the accuracy and efficacy of vanadium salts as potential drugs for the treatment of diabetes mellitus [55-59]. Diabetic patients with diabetes mellitus type 1 and 2 were treated with sodium metavanadate. The first group of patients did not show any amelioration in their glucose metabolism but they had lower insulin requirements. In patients with diabetes mellitus type 2, an increase in the non oxidative metabolism of glucose was observed. Total cholesterol decreased in type 2 diabetic patients and the side effects during that treatment were slight gastrointestinal disturbances [56].

Other clinical trials performed in type 2 diabetic patients with vanadyl sulfate showed a better hepatic response to insulin [55], a decrease in the levels of plasma glucose with the presence of slight gastrointestinal disturbances during the first week of treatment [58]. Besides, it was reported a decrease in the glycated hemoglobin (HbA1c) concentration in diabetic patents and an increase of the sensitivity of peripheral tissues to insulin action while no change in these parameters could be seen in a healthy controls [57, 60]. The last clinical trial carried out by Cusi *et al.* in Argentina displayed more encouraging results than the previous studies [59]. In this study, vanadyl sulfate (150 mg / day) was administered to 11 type 2 diabetic patients during 6 weeks. The treatment diminished the plasma level of glucose from 194 +/- 16 to 155 +/- 15 mg/dL. Besides, the glycated hemoglobin (HbA1c) and the fructosamine also decreased significantly and no change could be detected in the body weight of the patients. Vanadyl sulfate treatment decreased the endogenous glucose production by 20% ($P< 0.01$) an effect that correlated quite well with the lowering of fasted plasma glucose. Vanadyl also stimulated the insulin dependent utilization of glucose but, nevertheless, the increase towards insulin action did not correlate with the diminution in the fasted blood glucose levels. Moreover, the treatment decreased the total cholesterol and the LDL cholesterol concentration in the patients. The authors suggested that vanadyl sulfate in small doses increased the liver and muscle sensitivity towards insulin action in patients of type 2 diabetes mellitus. Decrease in endogenous glucose production correlated with the lowering of fasted plasma glucose but did not correlate with the insulin dependent utilization of glucose. These results suggested that the liver is more sensitive than the muscle to therapeutic doses of vanadyl sulfate in patients of type 2 diabetes mellitus.

At present, the first stage in clinical trials for a vanadium compounds has been accomplished [61, 62]. Phase I clinical trial of a designed vanadium complex, bis(ethylmaltolato) oxovanadium(IV) (BEOV) [63, 65] was completed [61, 62] and summarized in [38, 61]. The overall objective of this Phase I trial was to assess the safety and tolerability of BEOV. The results suggest that no adverse health effects were observed in any of the volunteers; gastrointestinal, liver and kidney function, as well as blood parameters all remained within normal levels throughout the study and the relative bioavailability of vanadium from BEOV was estimated to be three times that of an equivalent dose of vanadium from vanadyl sulphate [38].

As noted above, previous clinical trials in human beings have shown that vanadyl sulphate, 150 mg / day during six

weeks, seemed to be safe and they are relatively well tolerated by patients [59]. The studies did not show hematological changes or oxidative stress in the tissues. Nevertheless, some gastrointestinal disturbances were detected and this obliges one to limit the vanadium doses administered. The increment of the doses during the treatment showed a better tolerance [58, 59]. The majority of type 2 diabetes mellitus patients treated with vanadyl compounds showed an increase towards insulin sensitivity mainly through the stimulation of the non oxidative metabolism of glucose [56]. Reported results in human beings suggested that the normoglycemic effects of vanadium compounds may be dependent on insulin since they could behave as enhancers for the hormone action [55-57].

The putative mechanisms of action of vanadium compounds are currently under exhaustive investigation and at present it is possible to differentiate physiological and cellular responses and, on the other hand, molecular mechanisms. Up to now, as a response to cellular effects, different pathways seem to be involved in their ability to enhance glucose transport and metabolism, lipid, DNA and protein synthesis. Vanadium compounds have also mitogenic effects on different cell types [4, 5, 20, 40, 65-71]. *In vitro* studies have shown that both vanadate and vanadyl inhibit lipolysis and stimulate glucose oxidation, glucose transport, glycogen synthase and tyrosine phosphorylation in rat adipocytes [21, 72-74].

On the other hand, in experimental models of diabetes, in which diabetes mellitus type 1 is induced by streptozotocin injection, it is possible to demonstrate the physiological actions of vanadium compounds. These studies showed that 0.5 mg / ml of vanadate in the drinking water during 6 months normalized blood glucose levels in STZ-diabetic animals [22]. Another study (noted above) in the same model of diabetic animals showed that 0.8 and 0.2 mg / ml of sodium metavanadate reduced the hyperglycemia without causing hypoglycemia [23]. The rats that received the lower doses of vanadate reached a plasma vanadium concentration of 0.8 μg / ml after three weeks of treatment.

With the aim to elucidate which mechanisms may be involved in the euglycemic effects of vanadium in the STZ rats, cells from adipose, muscle and liver tissues were isolated to study the sensitivity towards the insulin. An increase in this parameter could be seen in adipocytes while the hepatocytes displayed a greater binding of insulin to its receptor and an increase in the glucose uptake could be seen in the muscle cells and hepatocytes [23]. It was also observed that in the liver of STZ rats, vanadate normalized the levels of mRNA of the glycolysis and glycogen synthesis related enzymes [12]. In another experimental model of diabetes, the ob/ob mouse, characterized by insulin resistant and hyperglycemia, treatment with oral 0.3 mg / ml vanadate during seven weeks, resulted in decreased plasma glucose, amelioration in the oral glucose tolerance test and also the induction of an early peak in the secretion of insulin [31]. In the same model, the administration of vanadate 0.25 mg / ml in the drinking water lowered plasma glucose from 2.36 to 1.43 mg / ml. On the other hand, in STZ rats treated with high doses of vanadyl sulphate it was studied the effect of this compound on the glycemic levels of the animals treated or not treated with exogenous insulin. These rats developed severe diabetes with levels of circulating insulin 25% lower than the control animals. The treatment with vanadyl started after the rats showed an important deficit of insulin. Vanadyl was able to reduce the hyperglycemia from 5.20 to 4.20 mg / ml. This means that it was not possible to normalize the glycemia of the diabetic rats. Nevertheless, when vanadyl was administered with insulin, it could be obtained a normalization of the glycemia with lower requirements for the hormone than the animals treated only with insulin [75].

The insulinmimetic actions of vanadium compounds described previously in this review are related to cellular and physiological responses, but the molecular actions seem to be based on the inhibition of different phosphatases since vanadate may, behave as a phosphate analog [76-80]. As a result of this structural similarity, vanadate might preserve the phosphorylation of the insulin receptor by irreversible binding and in this way it might regulate the signaling pathways initiated by insulin and other growth factors. Vanadium compounds may act by mimicking and/or circumventing the effects of insulin at the level of the insulin signaling cascade [73, 81-85]. In the insulin signaling cascade, insulin-induced tyrosine phosphorylation and activation of the β-subunit of its receptor result in the tyrosine phosphorylation of insulin receptor substrates (IRS), promoting the binding and activation of the lipid kinase PI3-K and through docking proteins and guanosine-nucleotide-binding protein, Ras, activate mitogen-activated protein kinase (MAPK). In particular, the extracellular regulated kinases-1,2 (ERK1,2). PI3-K activates PKB, also known as Akt (a product of Akt proto-oncogene). PKB, through phosphorylation and thereby inactivation of glycogen synthase kinase-3 (GSK-3), participates in glycogen synthesis [86]. Moreover, there is a cross talk between the MAPK pathway and PI3-K, generating a PI3-K/ras/ERK pathway which may play a key role in mediating the insulin mimetic effects of vanadium [87, 88]. ERKs are activated by various growth factors, which act through the receptor tyrosine kinases (RTKs), like platelet-derived growth factor (PDGF) [89, 90] and insulin -like growth factor-1 (IGF-1) [91]. Moreover, the ERK pathway is usually associated with cell proliferation and protection from apoptosis [92].

Studies performed in our laboratory showed the activation of ERKs by different factors and the participation of the PI3-K/ERKs transduction pathway both in apoptotic and antiapoptotic events; see below [39, 42, 49, 93-96]. On the other hand, vanadium has been shown to mimic several insulin-like effects *via* a staurosporine sensitive cytosolic protein tyrosine kinase, distinct from the insulin pathways [97, 98]. Studies carried out in osteoblasts in culture have shown the participation of this type of mechanisms to mediate the insulin mimetic effects [95]. In addition, the stabilization by vanadyl sulfate of the complex between insulin receptor and insulin receptor sustrate-1 [99], as well as the activation of other metabolic pathways [76, 100] may be contributing to the antidiabetic actions of the vanadium compounds.

VANADIUM OSTEOGENIC ACTIONS

Vanadium accumulates preferentially in skeletal tissue. Under conditions of dietary vanadium intake only, vanadium

is present in bones at concentrations ranging from 10-26 ug/g (10 - 26 μg / g) [54, 101-105].

Bone is a highly organized, metabolically active tissue consisting of a mineral phase of hydroxyapatite and amorphous calcium phosphate crystals deposited in an extra cellular organic matrix (ECM) [106]. The chemical nature and the open lattice of hydroxyapatite lend to substitution, both for the anions or the cations that form this mineral phase. Thus, the skeletal retention and bone effects of vanadium are of particular research interest in osteoporosis studies [107]. Bone is an active vanadium, and other metal ions, accumulator. It seems also likely that vanadium and strontium participate in the first stage of the mineralization process [108]. Vanadate ions can be incorporated into hydroxyapatite lattice, as phosphate analogs [109-111].

Since hydroxyapatite is the main mineral component of bones and hard tissues in mammals, the interaction of hydroxyapatite lattice formation with vanadium has been of special research interest. The incorporation of vanadium in synthetic apatite models showed that low or moderate VO_4^{3-} concentration did not produce any lattice distortion and had little effect on the strength of the P-O and O-H bonds [112]. Interchange of $[PO_4]^{3-}$ by $[VO_4]^{3-}$ is facilitated for the structural behavior of the hydroxyapatite lattice, which allows substitutions. Nevertheless, it is required that the apatite presents an amorphous form to allow the incorporation of vanadate since it could not be observed when the apatitic lattice is in the crystalline state. On the contrary, the substitution of calcium by other cations in the hydroxyapatite or fluoroapatite lattices affects these bonds more strongly [113,115]. Moreover, some work previously carried out to determine vanadium's biological role, suggested that diets deficient in vanadium might lead to thyroid gland and skeletal growth inadequacy [1, 115]. Besides, diets poor in vanadium content caused bone deficiency in goats [107]. We and others have previously demonstrated that vanadium compounds are insulin- and growth factor-mimetic compounds promoting bone formation and repair [4, 40, 68, 69, 96, 116]. Studies carried out using fibroblasts in culture demonstrated that vanadium compounds stimulated DNA and collagen synthesis, suggesting the role of vanadium as a promoter of osteoblast proliferation and differentiation [67, 68]. Even though the data are scarce in this context, vanadium could inhibit or impair bone resorption as a consequence of the inhibition of the Na,K-ATPase in bone [117]. Vanadium compounds also modulate osteoblastic differentiation, induce morphological alterations and stimulate the glucose consumption in these types of cells [94, 118, 119]. On the other hand, the alkaline phosphatase (ALP) activity is involved in the mineralization process of the bone. It has been shown that vanadium compounds inhibit ALP specific activity [4, 120-122].

Osteoblasts are one of the cell types that play an important role at bone formation. They produce the ECM where different types of bone related cells proliferate and differentiate, and where the mineralization process takes place. Recently, experiments in fish bone cells have shown that 10 μM metavanadate and decavanadate induced an increase in the cell proliferation but still affected the mineralizarion of the extracellular matriz [123].

Previous results obtained in our laboratory with osteoblast cells in short-term cultures with vanadyl complexes have shown interesting potential pharmacological actions [71, 124]. The complex with vanadyl(IV) and trehalose (TreVO) behaved as an osteogenic compound since it stimulated collagen production and caused a weak inhibition of ALP, a key enzyme for the mineralization process [40]. TreVO stimulated MC3T3E1 cell proliferation in the range of 5 - 25 μM in 24 h culture. For the long-term studies (up to 25 days), we used the lowest effective dose in the induction of cell proliferation (5 μM). The cells grew and proliferated until approximately 9 days of culture. Then the cell number remained constant through 25 days. After 10 days no significant differences in the cell number were observed for each culture condition. TreVO also produced an increase of collagen in these cells at 16 - 25 days of culture. TreVO enhanced collagen synthesis but it partially inhibited the ALP activity. After 30 days TreVO induced the formation of a great number of mineralization nodules in presence of an osteoblast cell differentiation inductor (mixture of ascorbic acid and β-glycerolphosphate) [125]. Besides, no cytotoxicity was observed after chronic incubation with the complex [40]. Altogether, these results suggest that the complex TreVO caused a positive effect on the MC3T3E1 cell differentiation and mineralization and behaved as an osteogenic drug.

In order to investigate the effects of vanadium in the hard tissues of animal models, obese and diabetic Zucker rats were treated orally with the complex of vanadyl cation with maltol, BMOV, (0,8 mg / ml in drinking water) and then the tibias and vertebras of the animals were examined [126]. This treatment improved the diabetic symptoms. Vanadium content in the bones of the treated rats was 9.4 μg / g in the tibia and 6.6 μg / g in the vertebras. Vanadium changed neither the levels of K, Mg, Na, Ca and P, nor the structure of the bones, as determined by X ray diffraction studies. As well, vanadium did not alter the architecture, mineral density and mechanical properties of bone. These results showed that the most important features of bone tissue were not altered by vanadium concentrations at which this metal exerts insulinmimetic actions [126].

More recently, two studies have been performed in order to investigate the effect of bis(ethylmaltolato)oxovanadium (IV) (BEOV) and vanadyl acetylacetonate (VAC) on the management of the diabetic state as well as on bone [127, 128]. Results suggested that BEOV improved the condition of bone, primarily by improving the diabetic state. BEOV also appeared to increase bone formation [127]. On the other hands, after administration of VAC, the decreased ultimate strength, trabecular thickness, mineral apposition rate, and plasma osteocalcin level in diabetic rats were either improved or normalized. Nevertheless, this treatment did not increase bone mineral density (BMD) in diabetic rats [128]. Both studies do not suggest that BEOV or VAC influence the bone of normal rats.

In the framework of a project devoted to the study of vanadium compounds with potential therapeutic application and in order to understand the involved mechanisms of action, we have carried out different experiments in cultured osteoblasts [71,125,129]. These studies have shown that at low concentrations, most vanadium derivatives behaved as

weak mitogens in comparison with insulin while at high concentrations they exerted cytotoxic effects. In general, we as well as other investigators have demonstrated that vanadium(V) compounds are more toxic than vanadium(IV) complexes. Vanadium derivatives also regulated osteoblastic differentiation, induced morphological alterations and stimulated the glucose consumption [118, 130]. Up to present, different efforts have been made to establish the mechanism of action of vanadium compounds in bone. Nevertheless, more experiments need to be done since currently this subject is poorly defined. As it has been mentioned above, MAPK and PI3-K are the main intracellular transduction pathways used by vanadium to exert its biological effects and these pathways are stimulated by vanadium compounds as a result of the inhibition by vanadium on PTPases [77, 78]. Nevertheless, some other mechanisms may also be involved in mediating the osteogenic effects of vanadium such as the direct activation of a cytosolic protein tyrosine kinase [74, 130]. Recent reported results from our laboratory are in agreement with the latter observations. Insulin and the vanadium(IV) complex with trehalose stimulated the glucose consumption in osteoblast-like cells, but the PI3-K inhibitor wortmannin did not abrogate this effect. Besides, staurosporine at concentrations that do not affect PKC, was a potent inhibitor of the glucose consumption simulated by this vanadium(IV) complex. [94]. On the other hand, one of the signalling phosphoprotein that modulates the insulin mechanism of action is glycogen synthase kinase-3 (GSK-3). The inhibition of GSK-3 (serine phosphorylated form) is important for the activation of glycogen synthase [132]. Vanadium(IV) complexes with sugars stimulated the phosphorylation of GSK-3. In the presence of staurosporine, the vanadium derivatives failed to stimulate GSK-3 phosphorylation [40]. Altogether these results suggest that the cytosolic tyrosine protein kinase and GSK-3 may be involved in the insulin mimetic activity of the vanadyl(IV) compounds on osteoblast cell in culture.

ANTITUMORAL PROPERTIES OF VANADIUM COMPOUNDS

Chemotherapy is the use of drugs to injure an invading organism without injury to the host. This definition therefore covers the antibacterial, antiviral and anticancer agents. In the first two, the invading organism is clearly distinct from the host. In the case of cancer, a family of diseases characterized by uncontrolled cellular proliferation, the organism is strictly not different but the treatment has a common aim, that of elimination of the unwanted cells. Thus, chemotherapeutic drugs must induce an irreversible cytotoxic effect. In the field of the application of Inorganic Chemistry to cancer therapeutics, by far the greatest success is the advent of cisplatin [133] and carboplatin [134] into the clinic. Metallopharmaceuticals have placed a relevant role in cancer treatment with cisplatin as a prototype. Even so, the development of analogs with structures similar to that of the cisplatin has produced many promising compounds. All direct structural analogs of cisplatin produce a very similar effect on target DNA and they induce similar biological responses. For this reason it is considered that the development of compounds of other metals structurally dissimilar to cisplatin may lead to a different spectrum of clinical activity. There are now many distinct classes of metal-based drugs with antitumor activity in experimental models. Another possibly exciting development is the recognition of certain ruthenium compounds as metastatic poisons rather than cytotoxic agents. Finally, the natural product bleomycin is always classified as an inorganic-based drug through the imputed DNA strand breakage mechanism facilitated by oxygen radical production, due to the presence of iron [135].

The antitumoral effects of vanadium compounds have been widely investigated on experimental animal models and various types of malignant cell lines. To perform the *in vitro* assays it is necessary to include cell models with non tumoral features in the studies with malignant cells, to be able to discard the toxic effects of the drug *per se* [40, 136, 137]. In particular, diverse vanadium compounds exert different effects on cell proliferation: sometimes they stimulate the mitogenesis but in another cases they are inhibitory agents of the cell cycle and also can inhibit cell proliferation even at the low doses [71, 138]. For this reason, some vanadium compounds may present antitumoral actions [7, 139].

Several mechanisms have been described to explain the inhibition of cell cycle or the induction of tumor cell death by vanadium derivatives. Important evidence supports the hypothesis that the antitumoral action of vanadium compounds is mediated by the inhibition of PTPases and the generation of reactive oxygen species (ROS) which in turn, cause a series of cellular effects such as DNA cleavage, PTPases inhibition and oxidative damage of different cellular components and organelles. PTPase inhibition regulates different transduction pathways which trigger apoptosis, cell cycle arrest and modulation of proteins involved in the metastatic ability of the tumors. On the other had, the interaction of vanadium with carcinogenic substances (i.e. alkyl drugs), may generate chemopreventive action. This behavior has been supported by experiments described in previous reports. Proliferation of the human tumoral cells (HTB-14), hematopoietic mouse cells (MDAY-D2) and endothelial mouse cells (EDMA) was inhibited by 5 - 50 μM of sodium orthovanadate. It has been also shown that the cells in an active proliferation state were more sensitive to the inhibitory effect of vanadium [140].

A series of specific PTPases such as SHP2 / SYP [141, 142] and dual phosphatases like cdc25 [143] involved in the promotion of cell proliferation have been identified and described. It has been suggested that vanadium compounds may be acting at these points of the cell cycle causing the inhibition of these types of phosphatases and, as a consequence, arresting cell proliferation. Vanadate [144] and pervanadate [145] caused the interruption of the cell cycle in G2/M phase. To progress to this point, the cell cycle requires the activation of the kinase complex dependent on cyclins CDK1 /cdc2-B cyclin which, in turn, is subjected to the dephosphorylation of cdc2 kinase by the cdc25 phosphatase. Recently it has been found that the peroxovanadates are potent inhibitors of the cdc25 phosphatase [146]. In addition, these vanadium compounds have been proven to be effective inhibitors of 28 tumoral cell lines as well as a breast tumor implanted in mice [146].

In another experiment, Shi and coworkers studied the relationship between the cell cycle and the generation of reac-

tive oxygen species (ROS) [147]. The incubation of epithelial lung cells with 100 μM vanadate generated hydrogen peroxide, hydroxyl and superoxide radicals, all ROS. Vanadate may induce an arrest of the cell cycle in the transition G2-M, which depends on the dose and the incubation time. This inhibitory effect seems to be mediated through the inhibition of PTPase cdc25 that controls the cycle [147]. Two mouse fibroblastic cell lines, one of them deficient in the suppressor tumor gene p53, showed also that vanadium induced an arrest of cell cycle at G2-M transition; however, in normal fibroblasts, the inhibition of the cycle seems to take place at phase S [148]. Interestingly, the blocking of cell cycle at the transition G2-M seems to be related to induction of cell death [149].

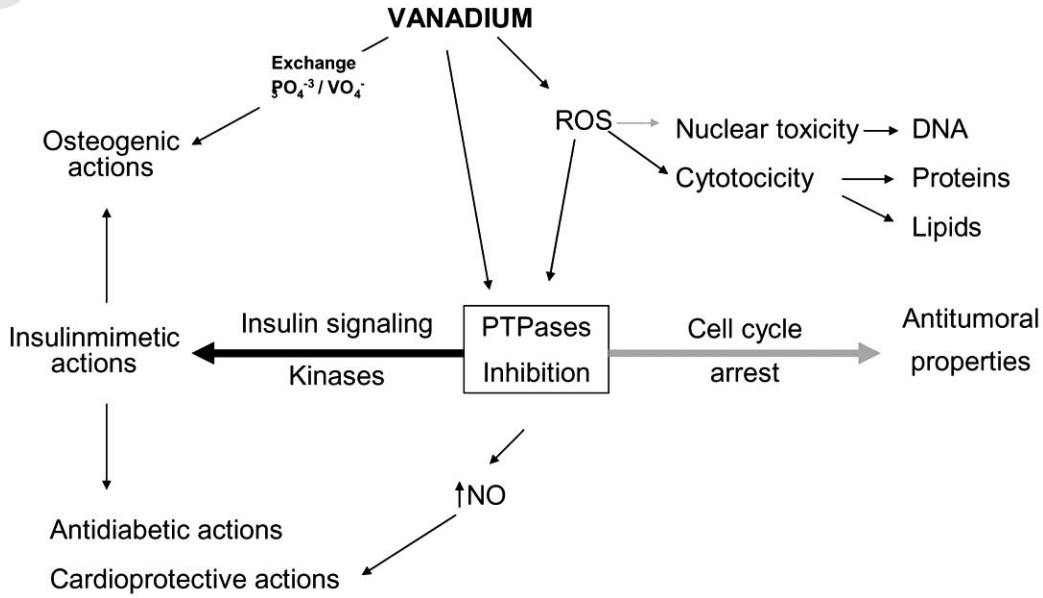
Metvan, bis(4,7-dimethyl-1,10-phenanthroine) oxovanadium (IV) sulfate, was one of the most promising synthesized compounds to enhance the antitumoral effects of vanadium. Metvan, at nano and micromolar doses, induced apoptosis in different human tumoral cell lines [150]. Moreover, it was more effective than cisplatin in the inhibition of ovarian and testicle tumoral cell. The mechanism underlying this antitumoral effect seems to be related to the induction of apoptosis [150].

Adhesions to the ECM, migration and colony formation or clonogenicity are the main mechanisms used by tumor types that form metastasis in the neighbor tissues. Different complexes of vanadyl(IV) with organic ligands of biological and pharmacological interest have been shown to be effective inhibitors of the aforementioned processes through a mechanism dependent on PKA [151]. Another antitumoral mechanism used by vanadium compounds could be related to the prevention of tumoral cell generation through the inhibition of the carcinogenic events of vanadium derivatives able to cause the alkylation of organic molecules in the cells (alkylating compounds) such as 7,12-dimethylbenzo[a] anthracene or N-methyl-N´-nitro-N-nitrosoguanidine or the antioxidant properties of some other vanadium complexes [152]. Experiments developed in rat models were useful in demonstrating the chemoprotective and chemotherapeutic effects to the animals, of vanadium, administered in the diet. [153-160].

CARDIOPROTECTIVE EFFECTS OF VANADIUM

Potential protective effects of vanadium compounds on the cardiovascular system have been studied in parallel with the insulinmimetic actions of vanadium. Nevertheless, it is worthy to mention that there has been a significant interest in this field, over the years [161-163]. Cardioprotective action of vanadium compounds seems to be related to their antihypertensive [164], hypocholesterolemic [56], hypoglycemic and hypolipidemic [29, 165] actions. Besides, the effects of vanadium derivatives as growth factor mimics and as antiapoptotic agents also may play an important role [4, 161, 165, 166] as well as its function on the inhibition of cardiac hypertrophy [167] and over the vascular cell contraction [168].

The above mentioned physiological and cellular effects of vanadium would be mediated through the inhibition of PTPases [99,169,170], the generation of nitric oxide (NO) [161,167,171,172] and the activation of the PI3-K/PKB pathway [162]. Considerable epidemiological, clinical and experimental data lend credence to the association between essential hypertension and abnormalities in carbohydrate and lipid metabolism [163,173]. In a series of experiments, Bhanot and co-workers employed vanadium compounds to examine the relationship between hyperinsulinemia, insulin resistance and hypertension [163,174,175]. In two models of hypertension (spontaneously hypertensive rat (SHR) and the fructose-hypertensive rat), Vanadyl (0.4 to 0.6 mmol/kg per day) prevented the rise in plasma insulin (treated, 211.2 +/- 6.0 pmol/L, $p < .001$) and blood pressure (treated, 127 +/- 3.0 mm Hg, $p < .001$). Intracellular transduction pathway PI3-K/PKB seems to be related with the myocardial hypertrophy [176, 177]. In a model of heart injury, vanadium compounds activated PKB and promoted cardiac remodeling



Scheme 1. Putative pathways used by vanadium compounds to exert pharmacological actions.

to recover the physiological contraction and relaxation of the cardiomyocytes. The inhibition of cardiac hypertrophy by bis(1-oxy-2-pyridinethiolato) oxovanadium (IV), (VO(OPT)), was closely associated with recovery of heart rate, mean artery blood pressure and contractile heart functions [167].

CONCLUSIONS AND PERSPECTIVE

Our current knowledge of the putative pathways underlying the pharmacological actions of vanadium compounds are summarized in the Scheme **1**.

The intricate relationships between the different pathways reveal the complexity of this field. In fact, some pathways trigger different biological responses (i.e. proliferation or cell cycle arrest) depending on the type and duration of the stimuli. Although some of these pathways deserved more and exhaustive research, many of them are currently quite well understood and allow a rationale design of new vanadium derivatives with potential applications in the treatment of different diseases. According to literature reports and our own experience, the activation of a determined cellular pathway depends on the cellular type and their metabolic status. For instance, depending on the redox potential inside the cells would predominate different oxidation states for vanadium (+5 in an oxidative environment and +4 in normal metabolic conditions). The oxidation state of vanadium also plays an important role in the target pathway used for a vanadium species to exert its biological or pharmacological effects, and finally as it has been shown, the nature of the ligand use to chelate the inorganic forms of vanadium causes different permeation and bioavailability of the vanadium compound with a logically different consequence for its pharmacological potency. For these reasons, it will be a subject of intense research in the near future to explore the mechanisms of action of different vanadium complexes in a great number of cell lines in order to understand the complexity of the pharmacological effects and to obtain a good structure-activity relationship to establish the rationale basis of vanadium pharmaceuticals design.

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