

Chapter 7

Vanadium Effects on Bone Metabolism

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Abstract Vanadium compounds are present in trace amounts in living organisms. In mammals, once absorbed from the gastrointestinal tract, they distribute among tissues and storage mainly in liver, kidney and bone. Vanadium compounds in pharmacological doses exert interesting effects as insulin enhancers, antitumoral and osteogenic agents. This review deals with the more relevant information about the osteogenic effects of vanadium compounds. Their actions on the different components of hard tissue and bone related cells in culture are summarized. Besides, the putative mechanism of action and the effects on in vivo models are also discussed.

Keywords Vanadium • Osteoblasts • Mechanism of action • Cytotoxicity • Genotoxicity

7.1 Introduction

Vanadium is a trace element present in biological systems, both in plants and animals. In vertebrates, it enters the organism mainly by the digestive and respiratory tracts through food and inhalation, respectively [1, 2]. Once absorbed, it distributes

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among tissues and accumulates in different organs, being the greater quantities of vanadium detected in liver, kidney and especially in bone [2].

While vanadium essentiality has been established in lower forms of life and also in higher plants, convincing evidence to support an essential role for this element in humans is still lacking [3].

Although ubiquitous in air, soil, water, and food supply, vanadium is generally found in nanogram or microgram quantities, which makes it difficult to measure. Its role as an essential element in humans remains uncertain due in part to the lack of symptoms associated to its deficiency in man and to the scarce methods strictly suitable to assure the absolute absence of this trace element in vanadium free diets for experimental animal models. However, there is scientific consensus on the putative biochemical role for vanadium compounds in living organisms [4].

In animal models and human beings, pharmacologic amounts of vanadium (i.e., 10–100 times normal intake) affect cholesterol and triglyceride metabolism, stimulate glucose oxidation and glycogen synthesis in the liver [5].

Vanadium's primary mode of action is as a cofactor that enhances or inhibits enzymes. The inhibition of the activity of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ by vanadate was the first effect of vanadium thoroughly investigated and reported in the literature [6], but currently, many other biological and pharmacological actions have been described for vanadium derivatives [7]. The role of vanadium in regulation of intracellular signaling pathways converts it in a possible therapeutic agent to be use in the treatments of a number of diseases. Different metabolic alterations for carbohydrates, lipids and proteins are markers for Diabetes mellitus. Besides, the disease is also characterized for a group of chronic complications which diminish the quality of life of diabetic patients and, finally, cause their death. Patients with Diabetes mellitus, mainly type 1 diabetic patients develop higher rates of bone resorption and turnover which convey to a decrease bone mineral density compared with healthy controls. Recently, Diabetes mellitus has been shown to be associated with osteoporosis [8, 9]. Several mechanisms seem to underlay bone alteration process in diabetic patients. Both, micro and macro angiopathies cause a decrease of blood flow especially in highly vascularised tissues. As a consequence, different metabolic changes take place in these tissues, including bone. For instance, it is probably that the synthesis and function of anabolic factors for bone development would decrease and, also highly concentrations of advanced glycation end (AGE) products can be formed in the constituents of the extracellular matrix (ECM). Among these components, collagen is one of the more affected proteins which cause a great impact in bone formation and functions [10]. Recently, some vanadium compounds have been reported in the literature as osteogenic agents due to their actions on bone related cells and on collagen formation. Besides, from a chemical point of view, it is well known the possible replacement of phosphorous by vanadium in the apatite lattice of bone tissue [11–14]. This review addresses and summarizes the osteogenic effects of vanadium compounds both *in vitro* and *in vivo* models as well as on cell regulatory processes that can be considered as putative mechanisms of action for these compounds. Besides, the possible mechanisms of adverse side effects derived from the vanadium administration are also briefly commented.

7.2 Hard Tissue

Bone is a dynamic tissue that is constantly being reshaped by osteoblasts, which build bone, and osteoclasts, which resorb bone. Osteoclasts and osteoblasts are instrumental cells in controlling the amount of bone tissue in vertebrates.

Osteoblasts are mononucleate cells responsible for bone formation. The origin of these cells is depicted in Fig. 7.1. Osteoblasts derive from osteoprogenitor cells located in the periosteum and the bone marrow. Progenitor osteoblastic cells are immature cells that express the crucial regulatory transcription factor *Cbfa1/Runx2*. Osteoprogenitors differentiate under the influence of growth factors, in particular the bone morphogenetic proteins (BMPs) [15]. Moreover, other growth factors including fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF- β) may promote the proliferation and differentiation of osteoblast precursors and potentially induce the mineralization process (osteogenesis). Mature osteoblasts are highly specialized cells which synthesize the major constituents of the osteoid, that is the unmineralized, organic extracellular matrix (ECM), which is composed mainly of Type I collagen and noncollagenous proteins. Osteoblasts are also responsible for mineralization of the osteoid. In fact, the mature osteoblasts promote the formation of the biological hydroxyapatite, the main hard tissue mineral phase component. These cells also regulate their content of mineral ions through the activity of different enzymes such as alkaline phosphatase (ALP) which is anchored in the osteoblastic membranes and plays a key role in the mineralization process. Aside from ALP, the organic matrix exerts a great degree of crystallographic control over the nucleation and growth of mineral particles.

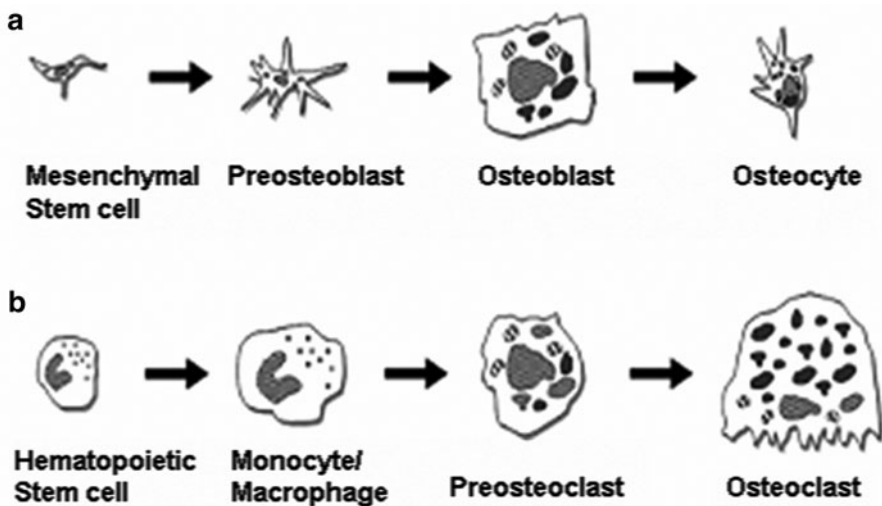


Fig. 7.1 Schematic origin of osteoblasts and osteoclasts

In the different steps of mineralization, type I collagen, the predominant protein in the ECM of bone and teeth, plays a key role [16]. Osteoblasts that are trapped in the bone matrix become osteocytes. They cease to generate osteoid and mineralized matrix, and instead act in a paracrine manner on active osteoblasts.

The other relevant cells in hard tissues are the osteoclasts. They remove bone tissue through a process known as bone resorption. Osteoclasts are multinucleate cells formed by the fusion of cells of the monocyte-macrophage cell line (Fig. 7.1).

Osteoclasts are characterized by high expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K [17].

7.3 Vanadium Compounds Interactions with Hard Tissue Components

7.3.1 With Hydroxyapatite

Hydroxyapatite is the major mineral component of the mineral phase of bones and hard tissues in mammals. It is thermodynamically more stable than other minerals which form during the development of the mineralization process. Hydroxyapatite is synthesized *via* intermediate precursors such as amorphous calcium phosphate (ACP), octacalcium phosphate, or dicalcium phosphate dehydrate [18–20].

The chemical nature and the open lattice of hydroxyapatite promote substitution events both for cations and anions (Fig. 7.2, Taken from reference [21]).

The most common substitutions involve carbonate, fluoride and chloride for hydroxyl groups, while defects can also exist resulting in deficient hydroxyapatite. The substitution and the vacancy effects are very important since these processes in the apatite lattice contribute to a greater solubility of this phase and in consequence, favor its behavior as an ion reservoir. Physiologically, the mineralization process takes place with a non random distribution. It occurs inside **matrix vesicles** (MVs) which are extracellular, membrane-invested particles selectively located at sites of initial calcification in cartilage, bone, and predentin [22].

Matrix vesicle biogenesis occurs by polarized budding and pinching-off of vesicles from specific regions of the outer plasma membranes of differentiating growth plate chondrocytes. The first crystals of apatite bone mineral are formed within the MVs [16]. This is followed by propagation of hydroxyapatite into the ECM and its deposition between collagen fibrils.

When vanadium is absorbed in vertebrates, it distributes among different tissues and is storage mainly in bone [23].

The skeletal retention and bone effects of vanadium are of particular research interest [24]. Vanadate ions can be incorporated into hydroxyapatite lattice possibly for its analogy to phosphate [25, 26]. For these reasons, special attention was focused on the interaction of vanadium species with hydroxyapatite and with some components of the ECM such as chondroitin sulfate A, N-acetyl-D-galactosamine

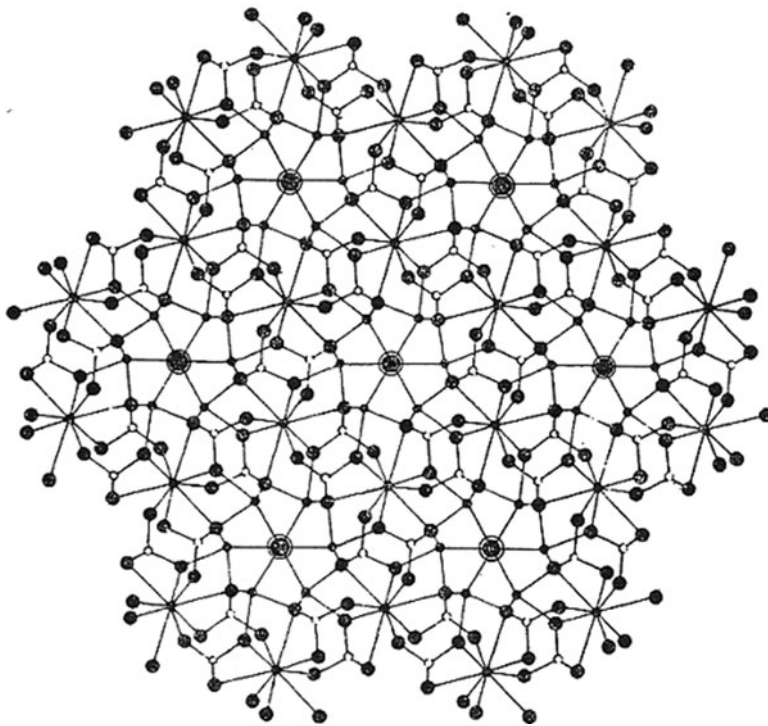


Fig. 7.2 Hydroxyapatite lattice. Taken from reference [21]

and D-Glucuronic acid. The structural and spectroscopic effects of the incorporation of VO_4^{3-} in the hydroxyapatite lattice were investigated as a model for the process of incorporation of vanadium in bone [27]. The substitution of PO_4^{3-} by VO_4^{3-} is facilitated for the structural behavior of the hydroxyapatite lattice which lends itself for substitution. Nevertheless, it is required that the apatite would be in an amorphous form for the incorporation of vanadate to be accomplished since it could not be observed when the apatite lattice is in the crystalline state. The incorporation of small quantities of VO_4^{3-} in the PO_4^{3-} sites produces only slight distortions in the macroscopic and microscopic structure of the mineral phase of bones. This fact could be determined by X-ray diffraction studies of substituted hydroxyapatite powder samples. Based on these results, it can be assumed that bone has an active role in the detoxification process when the organisms are exposed to high vanadium concentrations, comparable to that known for other toxic trace elements that can be also incorporated in bone. On the contrary, the substitution of calcium by other cations in the hydroxyapatite lattice affects these bonds more strongly [28, 29]. Besides, it was also demonstrated that VO^{2+} could not be incorporated into the apatite lattice although it is strongly adsorbed on its surface [30]. All together these studies showed that hydroxyapatite is a good model to study the incorporation of vanadium in bone.

7.3.2 With ECM Components

To continue with the understanding of the effects of vanadium species on hard tissues we investigated the interactions of vanadium with chondroitin sulfate A (CSA), an acid mucopolysaccharide present in connective tissue and other mineralized systems [31]. Results from different spectroscopic determinations suggested the coordination of oxovanadium(IV) to the carboxylate group and the glycosidic oxygen of the D-glucuronate moieties [32]. These results are relevant for the interaction of VO^{2+} with collagen, a system in which interactions of oxovanadium(IV) with nitrogen has been clearly established [33, 34].

7.3.3 With Bone Cells in Culture

The relevance of vanadium in bone tissue arises from the studies performed to establish the essentiality of this element in animals and human beings [23, 35].

As bone is quantitatively the main tissue for vanadium storage bone accumulation is twice than the accumulation in kidney and tenfold the liver accumulation [36] it is worthy to try to understand the effects of vanadium compounds in bone related cells. Cells in culture are a useful system to investigate many different biological events. In particular, considering the biology and biochemistry of hard tissues, it is interesting to thoroughly study events such as cell proliferation, differentiation and mineralization, as well as the intracellular mechanisms by which vanadium derivatives exert their biological actions.

In particular, we have studied the effects of vanadium compounds in two osteoblast-cell lines of murine origin Fig. 7.3. [37–40].

MC3T3E1 osteoblasts are derived from mouse calvaria. These cells display the features of typical fibroblasts [41].

MC3T3E1 cell line is a model of preosteoblasts that can differentiate to mature osteoblasts in culture. The different maturation stages of this cell line *in vitro* resemble that of the physiological process *in vivo* (proliferation, differentiation and mineralization), providing an adequate system for biological studies referred to bone tissues.

In the proliferative stage, MC3T3E1 cells do not express a great level of alkaline phosphatase (ALP) specific activity. After 10 days of culture in the presence of ascorbic acid and β glycerophosphate, they expressed specific markers of mature osteoblast phenotype. The differentiation step correlates with the expression of different proteins such as ALP and collagen accumulation in the ECM. The mineralization of ECM begins approximately at 15–20 days of culture. After that, the cells programme their death by apoptosis [42].

UMR106 osteoblast-like cells are derived from a rat osteosarcoma induced by 32P. This immortalized cell line exhibits the characteristic osteoblast phenotype. The cells express high levels of specific ALP activity and produce type I collagen but they are unable to synthesize bone mineral phase in culture [39].

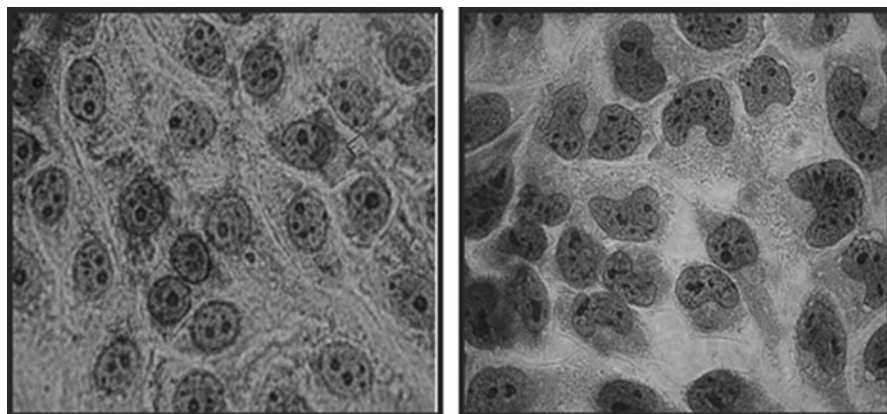


Fig. 7.3 MC3T3E1 osteoblast-like cells. The osteoblasts were cultured in DMEM at 37°C for 24 h, fixed and stained with Giemsa for microscopy observation (Magnification: 100×) (*Left* panel). UMR106 osteoblast-like cells. Osteoblasts were cultured in DMEM 37°C, 24 h, fixed and stained with Giemsa for microscopy observation (Magnification: 100×) (*Right* panel)

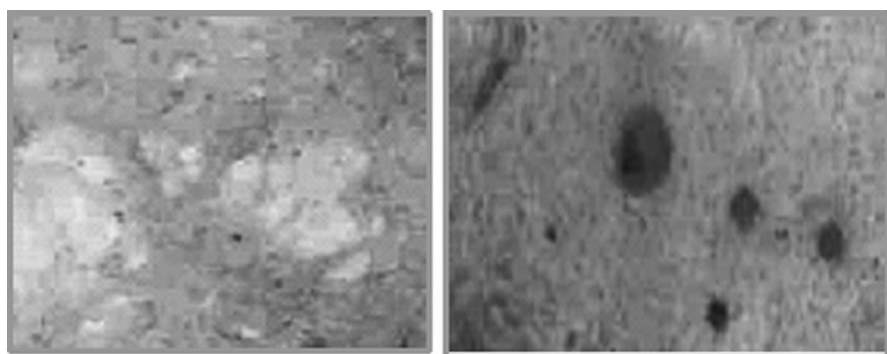


Fig. 7.4 Effect of TreVO long-term treatment on the mineralization of MC3T3E1 cells. Control cells (*Left* panel). Long-term treatment with 5 μ M TreVO (*Right* panel)

This simple model of osteoblast-like cells in culture allows the investigation of pharmacological effects of many vanadium compounds. Specially, complexes of vanadyl(IV) cation with simple sugars (mono- and disaccharides, and related compounds such as polyols and acids) were investigated [43, 44].

Practically all the vanadyl(IV)-sugar complexes synthesized in our laboratory were evaluated for their bioactivity on osteoblast cell lines in culture. This approach has allowed us to find an outstanding compound among this series: the complex of vanadyl(IV) cation with the disaccharide trehalose (TreVO) which displayed insulin mimetic activity [45]. Besides, in long term cultures carried out with the MC3T3E1 cell line [40], this complex revealed as a good osteogenic compound since it promoted type I collagen production and the mineralization of the ECM in the cultures (See Fig. 7.4) [40]. Moreover, it could also be established the mechanism of

action of this compound which exerted its mitogenic effect, at low doses, through the activation of the extracellular regulated kinase (ERK) pathway. On the other hand, it was demonstrated the participation of oxidative stress in the cytotoxic actions of this complex at high concentrations [39, 40].

Moreover, the bioactivity of vanadyl(IV) derivatives with flavonoids and related compounds have been also investigated with the aid of this cellular *in vitro* model to assess their osteogenic ability. In this context, a complex of vanadyl(IV) cation with the flavonoid Quercetin, also showed promissory osteogenic activity in osteoblasts in culture. Flavonoids are polyphenolic compounds obtained from plants. Recently they have aroused a great scientist interest due to their broad pharmacological activity. They present antioxidant, antitumoral and antibacterial properties [46–49].

The complex of Quercetin and vanadyl(IV) cation, QuerVO, was studied in cultures of MC3T3E1 and UMR106 cells. Cell proliferation was evaluated by the crystal violet bioassay. For studying cellular differentiation, two markers of osteoblast phenotype (ALP and collagen production) were analyzed. Finally, to get an insight into the putative mechanisms of action, studies on the effect of this complex on the activation of ERK pathway were performed and reported [50]. QuerVO displayed interesting osteogenic actions such as induction of the synthesis of collagen type I. The complex caused stimulation of ERK phosphorylation and this activation seems to be one of the possible mechanisms used by the complex to exert its biological effects.

Another interesting osteogenic oxovanadium (IV) derivative thoroughly studied for our group was the vanadyl(IV) complex with ascorbic acid (VOAsc) [51]. This complex significantly stimulated collagen production in osteoblasts and inhibited ALP activity in UMR106 cells. Besides, after 3 weeks of culture, VOAsc (5–25 μ M) increases the formation of mineralization nodules in the ECM of MC3T3E1 cultures. At higher concentrations this complex stimulated cellular apoptosis in osteoblast cell lines.

On the other hand, the effects of vanadium(V) compounds such as metavanadate and decavanadate in bone fish cells have been investigated by Aureliano Alves and his group [52]. Short- and long-term studies were performed in VSa13, a fish bone-derived cell line. Metavanadate in short periods was less toxic than decavanadate. In long term studies the effects of both vanadium derivatives were similar. They stimulated cell proliferation but strongly impaired the mineralization process.

The reported results suggest that vanadium(IV) complexes display more effective osteogenic properties in osteoblasts in culture than vanadium(V) derivatives, although more research is required in this sense to get a deeper insight into the osteogenic properties of vanadium.

As bone homeostasis is the result of the balance between bone resorption and bone formation, we have studied the activation of macrophages because these cells are related with bone resorption. We investigated the effect of a complex of vanadyl(IV) cation with the non steroidal anti-inflammatory drug Aspirin, VOAspi, on a culture of murine macrophage RAW 264.7. VOAspi caused the activation of macrophages by a mechanism dependent on L-type calcium channel and the generation of nitric oxide. On the contrary, free vanadyl(IV) cation exerted cytotoxic

effects by a mechanism independent of calcium channel and nitric oxide generation [53]. The studies on vanadium actions in osteoclasts and related cells are very scarce and merits more attention in the future.

7.4 Mechanism of Action

The putative mechanisms for the osteogenic actions of vanadium compounds are currently under exhaustive investigation. In fact, we have carried out different experiments in osteoblasts in culture to achieve this aim [37, 40, 54]. As an overview, at low concentrations, most vanadium derivatives behave as weak mitogens and promote osteoblast differentiation in a way similar to the insulin. Vanadium derivatives regulate osteoblastic proliferation, differentiation and stimulated the glucose consumption [55, 56]. Extracellular regulated kinases-1,2 (ERKs) and phosphatidylinositol-3 kinase (PI3-K) are the main intracellular transduction pathways used by vanadium to exert its biological effects. These pathways are stimulated as a result of the inhibition that vanadium derivatives cause on the protein tyrosine phosphatases (PTPases) [57, 58].

The model of osteoblastic cells in culture has allowed us to demonstrate the ability of low doses of TreVO to promote cell proliferation and to stimulate ERK phosphorylation in the MC3T3E1 osteoblastic cell line. This effect was totally abrogated by an inhibitor of MEK (PD98059) and wortmannin (an inhibitor of the PI3-K), but not by a mixture of free radical scavengers (vitamins E and C) [45]. These results suggest that low doses of the complex, which are mitogenic for MC3T3E1 cells, could act through the PI3K-MEK-ERK pathway and by a mechanism independent of free radicals. On the contrary, at higher doses, the vanadium complex inhibited cell proliferation of MC3T3E1 osteoblasts and the osteosarcoma UMR106 cell line. This effect was not blocked by neither wortmannin, nor PD98059 or by the mixture of vitamins C and E. However, high concentrations of the complex strongly increased ERK phosphorylation, an effect that was partially blocked by wortmannin, PD98059 or a mixture of vitamins E and C. In addition, the combination of these inhibitors showed an additive effect over the inhibition of ERK activation but not over the inhibition of cell proliferation [45]. Altogether these results indicate that, although high doses of vanadium stimulate ERK phosphorylation through the PI3K-MEK-dependent pathway and also through an oxidative mechanism, the inhibition of cell proliferation does not seem to be associated with the activation of these pathways.

On the other hand, there are some other mechanisms that may be involved in the osteogenic effects of vanadium such as the direct activation of a cytosolic protein tyrosine kinase [55, 59]. Results reported by our laboratory are in agreement with the latter observations. Insulin and TreVO stimulated the glucose consumption in osteoblast-like cells, but the PI3-K inhibitor wortmannin did not abrogate this effect. In addition, staurosporine at concentrations that do not affect PKC, was a potent inhibitor of the glucose consumption simulated by this vanadium(IV) complex.

[45]. In addition, the inhibition of glycogen synthase kinase-3 (GSK-3) is important for the activation of glycogen synthase [60]. Vanadium(IV) complexes inhibit GSK-3 through phosphorylation and in turn activate glycogen synthase promoting glucose consumption on osteoblasts. In the presence of staurosporine, the vanadium derivatives failed to stimulate GSK-3 phosphorylation [44]. 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.

7.5 *In Vivo* Osteogenic Effects of Vanadium

Several studies suggest that vanadium compounds ameliorate diabetic-related bone disorders, primarily by improving the diabetic state [61, 62].

Treatment in control and streptozotocin-induced diabetic female Wistar rats with bis(ethylmaltolato)oxovanadium(IV) (BEOV), was effective in incorporating vanadium into bone. In all treated groups, BEOV increased osteoid volume. In non-diabetic rats, BEOV increased cortical bone toughness, mineralization and bone formation [61] (Fig. 7.5).

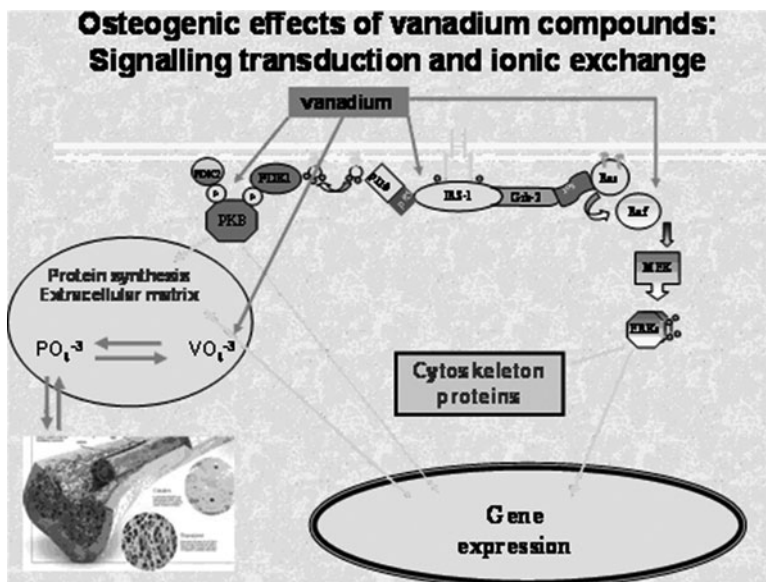


Fig. 7.5 In this figure it can be observed two of the most important signaling transduction pathways of insulin as well as the sites used by vanadium as an insulin enhancer. Both pathways regulate the expression of genes related to bone cell proliferation and differentiation as well as their action on proteins of the cytoskeleton which are related to cellular morphological changes. Moreover, vanadium compounds participate in ionic exchange with phosphate favoring its accumulation in bone tissue and potentiate effects on this tissue

Following the long-term administration of vanadyl acetylacetonate, trabecular thickness, mineral apposition rate and plasma osteocalcin in diabetic rats were either improved or normalized, however reduced bone mineral density was not increased [62].

In higher animals, vanadium has been demonstrated to be essential. After their mothers had been fed carefully formulated vanadium-deficient diets, second-generation goat kids suffered skeletal damage and died within 3 days of parturition [5]. In human beings vanadium essentiality has not been proven yet. Pharmacologic amounts of vanadium (i.e., 10–100 times normal intake) affect cholesterol and triglyceride metabolism, influence the shape of erythrocytes, and stimulate glucose oxidation and glycogen synthesis in the liver [5]. Moreover, clinical studies carried out in normal or diabetic patients indicated that orally administered vanadium(IV) showed low or no toxicity [63–65].

7.6 Risk Assessment for the Use of Vanadium Compounds as Potential Therapeutics Drugs: Cytotoxicity and Genotoxicity Studies

Despite the potential use of vanadium compounds either as insulinomimetic, osteogenic or anticancer drugs, its possible harmful effects argue against its clinical use. Understanding of the mechanisms underlying vanadium compounds toxicity is important in evaluating its use as a therapeutic drug.

The toxic effects of vanadium are related to the dose, cell line and vanadium nature (oxidation state, free or complexed). Studies concerning the mechanisms of action of vanadate showed that this compound induces gene expression, oxidative burst, changes in cytosolic calcium, tyrosine phosphorylation, enzyme activation or inhibition and also, morphological changes and cytoskeletal alterations [6, 66–71].

Several studies suggest that hydrogen peroxide is involved in vanadate-induced cell growth arrest and cell death [72–75].

However, a more recently study suggested that vanadate toxicity occurs by two distinct pathways, one dependent on and one independent of H_2O_2 production [76]. Vanadate concentrations that reduced cellular viability to approximately 60–70% of the control (10 $\mu\text{mol/L}$) did not induce H_2O_2 formation. A second hypothesis raised by these authors proposed that peroxovanadium compounds, produced once vanadate enters into the cells, are responsible for the cytotoxicity.

On the other hand, decreased cell viability induced by vanadyl sulphate in tumorigenic and non-carcinogenic cells has been also previously reported [77]. Moreover, we have also previously reported the cytotoxicity of a vanadium(V) derivative with salicylaldehyde semicarbazone in osteoblasts in culture [78]. This complex caused cytotoxicity in the osteoblastic cells mainly through oxidative stress and through the activation of ERK pathways which, although it is classically recognized as a key transducer in the signal cascade mediating the cell proliferation, and differentiation

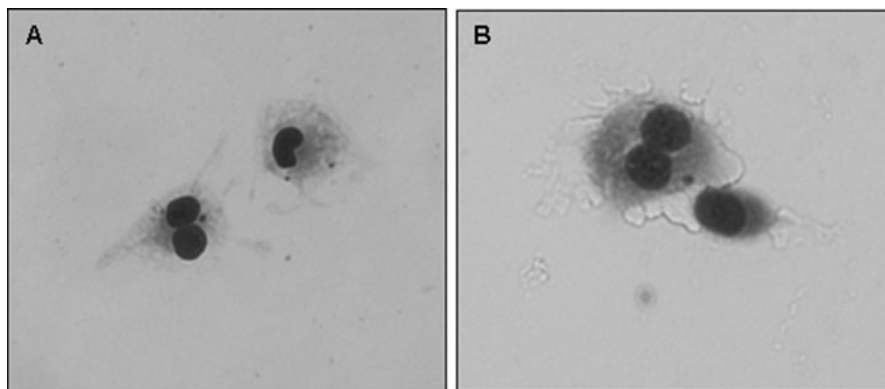


Fig. 7.6 Genotoxicity of VO(oda) in UMR106 cells (a) and MC3T3E1 osteoblasts (b). This picture shows the induction of micronucleus in binucleated cells stained with Giemsa (Magnification: 40 \times)

[79], some investigations have shown that the activation of ERK pathway can mediate cell cycle arrest and apoptotic and non apoptotic cell death [80].

On the other hand, even though vanadyl(IV) is less toxic than vanadium(V), it is always crucial to investigate the toxicity of vanadium(IV) derivatives intended to be used in therapeutics. Recently, we have reported an exhaustive study on a vanadyl(IV) complex with a multidentate oxygen donor oda=oxodiacetate, VO(oda) in osteoblast cell lines in culture [81]. The complex increased the level of ROS which correlated with a decreased in GSH/GSSG ratio, dissipation of the mitochondria membrane potential and promoted an increase in ERK cascade phosphorylation, which is involved in the regulation of cellular death and survival.

Considerable attention has attracted the assessment of the genotoxicity of vanadium compounds in recent years. Adverse effects of pentavalent and tetravalent vanadium compounds on chromosome integrity and segregation have been observed *in vitro*. In particular, we have studied the genotoxic effect of VO(oda) in two osteoblast cell lines (UMR106 and MC3T3E1) that revealed a twofold increase in the micronucleus frequency at 25 μ M compared with control in UMR106 and at 10 μ M in MC3T3E1. An example of a binucleated osteoblastic cell containing a micronucleus is depicted in Fig. 7.6 for both cell lines. Besides we have recently reported preliminary results on the increase in DNA strand breaks in a human colon adenocarcinoma cell line (Caco-2 cells) induced by VO(oda) [82]. In agreement with our results, vanadium(IV) compounds proved to be genotoxic in several *in vitro* systems, inducing micronuclei and chromosomal aberrations in human peripheral blood cells [83–85], and DNA strand breaks through the generation of free radicals [86, 87].

Pentavalent vanadium compounds have been also reported to induce genotoxic actions stimulating the increase in micronucleus frequency, DNA strand breaks, sister chromatid exchanges (SCE) and chromosomal aberrations in different *in vitro* systems [83, 84, 88–91].

These positive findings were confirmed in some *in vivo* studies in mice using acute intraperitoneal or intragastric administrations [92–94].

Moreover, the genotoxic potential *in vivo* by a subacute oral exposure of vanadyl sulphate has been evaluated. Effects on chromosome integrity and segregation, visualized as micronuclei, and primary DNA lesions detectable by comet assay, have been assessed in somatic and germ cells [95]. In summary, taken in consideration the multiple biological activities exerted by vanadium on critical cell mechanisms, even in the very low dose range [96, 97], it is suggested to maintain a cautious approach in the safety evaluation of vanadium compounds.

7.7 Concluding Remarks

The potential therapeutics actions of vanadium compounds at hard tissues have attracted the attention of scientists in the field of diabetes mellitus, bone disease, aging and cancer since vanadium compounds are store mainly in bones. The use of osteogenic primary or cloned lines in culture is a useful strategy to investigate the osteogenic properties of vanadium compounds. Although *in vitro* experiments showed that the toxicity actions of vanadium compounds seem to be related to the oxidation state of the element as well as the nature of the ligands in the coordination sphere and also, on the cell type in culture, *in vivo* reports show little, if any, toxicity in long-term administration. Vanadium compounds have demonstrated several interesting effects on biological processes such as cell proliferation, differentiation and mineralization of the ECM in bone cells. These effects convey to vanadium consideration as a potential therapeutic agent for several diseases. Finally, it is worthy to mention that further investigations are needed to completely elucidate the osteogenic mechanism of action of vanadium compounds, even though, currently most evidence point to the role of ERK pathway activation through the inhibitory effect on Protein tyrosine phosphatase (PTPase) activity, calcium channel and the expression of genes related to osteoblast differentiation. Moreover, although there are very few experimental works on this topic *in vivo*, some studies recently develop suggest the potential relevance of vanadium derivatives in the treatment of bone diseases.

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