

# Vanadium in cancer treatment

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Accepted 28 June 2001

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## Abstract

Vanadium compounds exert preventive effects against chemical carcinogenesis on animals, by modifying, mainly, various xenobiotic enzymes, inhibiting, thus, carcinogen-derived active metabolites. Studies on various cell lines reveal that vanadium exerts its antitumor effects through inhibition of cellular tyrosine phosphatases and/or activation of tyrosine phosphorylases. Both effects activate signal transduction pathways leading either to apoptosis and/or to activation of tumor suppressor genes. Furthermore, vanadium compounds may induce cell-cycle arrest and/or cytotoxic effects through DNA cleavage and fragmentation and plasma membrane lipoperoxidation. Reactive oxygen species generated by Fenton-like reactions and/or during the intracellular reduction of V(V) to V(IV) by, mainly, NADPH, participate to the majority of the vanadium-induced intracellular events. Vanadium may also exert inhibitory effects on cancer cell metastatic potential through modulation of cellular adhesive molecules, and reverse antineoplastic drug resistance. It also possesses low toxicity that, in combination with the synthesis of new, more potent and better tolerated complexes, may establish vanadium as an effective non-platinum, metal antitumor agent. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords:* Vanadium; Biochemistry; Anticancer effects; Apoptosis; Free radicals; Metastasis; Toxicity; Drug resistance

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## 1. Introduction

Vanadium is a trace element known to be essential for a number of species that is widely distributed on earth. However its role as a micronutrient in humans has yet to be established.

Humans and other species may be exposed to the metal through the atmosphere, polluted from the combustion products of vanadium-bearing fuel oils, fumes and dust generated from metallurgical refining and by food [1]. Foods contain low vanadium concentrations (below 1 ng/g), but the estimated daily intake of the US population ranges from 10 to 60  $\mu\text{g}$  [2]. Vanadium enters the organism by inhalation, the gastrointestinal tract and the skin and it is specifically stored in certain organs mainly in the liver, kidney and bones [3–5].

Although speculation that metal complexes may possess antitumor activity has existed since the beginning of the 20th century and survey of the antitumor action of inorganic compounds appeared in 1931, indications for the antineoplastic effects of vanadium salts was first shown in 1965 [6] whereas later in 1967, vanadium, tested among other metals against spontaneous mice tumors, was found to be inactive [7]. Metal antitumor activity received strong attention after the discovery of the anticancer effects of *cis*- $\text{PtCl}_2(\text{NH}_3)_2$  (*cis*platin) and research on vanadium was stimulated by the findings of English et al. [8], on the role of the metal as an inhibitor of terminal differentiation of murine erythro leukemia cells and by Thompson et al. [9], on the inhibition, by dietary vanadyl sulfate, of chemically induced mammary carcinogenesis. The latter also, provided evidence that this trace metal, which is typically present in the western type diet (at  $\mu\text{M}$  wet weight concentrations), may be an effective chemopreventive agent. Furthermore, the biological effects of vanadium compounds such as the insulin-like action [10,11] and the reduction of hyperlipidemia and hypertension, in relation to their few adverse effects [12], indicate the potential therapeutic applications of these compounds. Accumulating evidence from experiments carried out the last 15 years, suggest that vanadium could be considered as a representative of a new class of non-platinum, metal antitumor agents.

## 2. Chemical and biochemical properties of vanadium compounds in relation to cancer treatment

Vanadium is a group 5d transition metal possessing certain chemical and biochemical properties. A variety of vanadium compounds have been synthesized so far, in an effort to offer better tolerance, more potent action, better selectivity and less toxicity in cancer treatment. Chemical and biochemical characteristics determine the anticancer effects of vanadium compounds.

### 2.1. Vanadium chemistry and biochemistry

Vanadium, is a metal possessing a well-defined geometry of its inner coordination sphere, regarding to the usual oxidation states of the metal [V(III), V(IV) and V(V)], while its outer sphere (moieties) is not subject to such limitations. There is, thus, a donation of an electron pair from ligands of the inner sphere to the metal, conferring a high degree of covalent character to the resulting bond. Vanadium complexes with rapid exchange of donor atoms in a coordination sphere are characterized as ‘labile’; in contrast to those lacking easy atom movement which are characterized as ‘inert’. Vanadium (V) in the presence of peroxides and some polydentate ligands forms crystalline peroxo–heteroligand complexes. Heteroligands of these compounds can shift the redox potential of V(V)/V(IV) towards the point where an intramolecular electron transfer could occur within the [V(V)–peroxo] moiety, resulting to a weakening of V–O bonds toward coordinate dioxygen ligand and to the generation of superoxide ion [13].

A relationship has been found between the chemical composition and the antitumor activity and toxicity of peroxovanadates; this was shown to depend upon the type of the heteroligand [14]. At non-toxic concentrations the identity of the ancillary ligand of the peroxovanadium complex may play a significant role on the cell cycle arrest exerted by these complexes on tumor cell lines [15]. Concomitant treatment with vanadate (IV) and peroxide ( $\text{H}_2\text{O}_2$ ) enhances the biological effects of the metal in various cell lines, due probably to the formation of peroxovanadate. It was shown that treatment of cancer cell lines (rat hepatoma, rat basophilic leukemia, murine muscle and Chinese hamster ovary cells) with vanadate (V) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) markedly increased protein tyrosine phosphorylation and phosphoinositide breakdown, in a dose–response relationship with vanadate [16] and probably selectively inhibited protein tyrosine phosphatase activity in vitro [17]. Further, studies on 12 peroxo–vanadium complexes related the entering of peroxide ion ( $\text{O}_2^{2-}$ ) in the coordination sphere of vanadium, to the activation of the insulin receptor kinase and to the inhibition of the phosphotyrosine phosphatase in cultured hepatoma cells [18].

In the crystalline state the inner coordination sphere of peroxovanadium complexes assumes one of the well-defined polyhedral geometries such as tetragonal or pentagonal bipyramidal structure [15]. Polyhedral coordination of vanadium compounds, in different biomedica, is probably not existing at low vanadium concentrations, but may also play a role in the mechanical destruction of cancer cells [7].

Vanadocenes are organometallic complexes containing vanadium (IV), belonging to the metallocene class of antitumor agents [7,19–23]. These organometallic

complexes, with the vanadium (IV) linked to organic ligands by direct carbon metal bonds, exhibit in vitro and in vivo significant antitumor properties [7,19–27]. The vanadocenes containing vanadium (IV) are ‘bent sandwich’ complexes; bischloropentadienyl (CP<sub>2</sub>) moieties are positioned in a tetrahedral symmetry and in bent conformation with respect to the central metal atom [28,29]. The most promising among the various non-platinum metallocenes, apart from titanocene dichloride, is vanadocene dichloride [(C<sub>3</sub>H<sub>5</sub>)VCl<sub>2</sub>] [7,19,21,23].

Vanadium exists in aqueous solution as tetravalent (IV) vanadyl (VO<sup>2+</sup>) and pentavalent (V) vanadate (HVO<sub>4</sub><sup>-</sup>, VO<sub>3</sub><sup>-</sup> and/or H<sub>2</sub>VO<sub>4</sub><sup>-</sup>) [13,15,30]. A number of monomeric and polymeric tetravalent [V(IV)] and pentavalent [V(V)] vanadium species can be present in aqueous solutions, their composition depending upon pH and vanadium concentrations.

In presence of oxidizing agents the vanadium ion is present as the hydrated monomer of vanadate (HVO<sub>4</sub><sup>2-</sup> or H<sub>2</sub>VO<sub>4</sub><sup>-</sup>) at micromolar concentrations near neutral pH, whereas in the presence of extracellular or intracellular reducing agents (e.g. glutathione), the anion is reduced to the cation vanadyl (VO<sub>2</sub><sup>+</sup>), within a few minutes [14,31–35]. Bioreduction of vanadium (V) to vanadium (IV) by glutathione [36] or by microsomes through hydroxyl radicals generation [37] as well as by NADH-dependent flavoenzymes [38] seems to regulate various cellular actions of vanadium compounds such as its cytotoxic and morphological effects on various cells. Vanadate also begins to polymerize at concentrations greater than 0.1 mM at neutral pH [30]. The monomer and polymers V(V) species up to the hexamer, undergo rapid polymerization and depolymerization with pH changes, whereas the decamer is formed rapidly when a V(V) solution is acidified but only slowly depolymerized when the solution is brought back to neutral pH. There is evidence indicating that V(V) at different states of polymerization may also have different toxicity [39]. Vanadium may also exist at low oxidation states as trivalent [V(III)] and divalent [V(II)] ions [33]. Vanadium existing at trivalent state [V(III)] as in two newly synthesized complexes with the sulfhydryl-containing pseudopeptide *N*-(2-mercapto-propionyl)glycine and the amino acid cysteine, is slowly oxidized to V(IV) in aqueous solutions [34,35,40–42]. At physiological pH vanadium is found in the pentavalent oxidation state, as vanadate anion, which is the most stable form among its various oxidation states [43,44]. The oxidation state of vanadium in vitro and in vivo is related to a variety of its biological effects among which is the participation in free radical reactions; these are involved either to its potential carcinogenicity and/or to its regulatory and toxic effects on normal and cancer cells and in animals (Section 4.3).

The oxidation state of vanadium seems also to determine some differences in biological effects of vanadium compounds. Vanadate (V) and oxovanadium (IV) complexes are, for example, both inhibitors of protein phosphotyrosines (PTPs) in many cells, acting however, with different mechanisms. Vanadate appears to act as a phosphate analogue mimicking the transition state [45–49]. Thus vanadate forms a weak and reversible bond with the thiol group of the PTPs, acting as a competitive inhibitor. Peroxovanadium complexes and aqueous peroxovanadates are however irreversible and more potent PTPs inhibitors since the critical cysteine residue in their catalytic domain is oxidized by peroxovanadium complexes [15,46]. The widely used PTP inhibitor pervanadate is a peroxovanadium compound generated by reaction of H<sub>2</sub>O<sub>2</sub> with orthovanadate, in vivo and in vitro. Pervanadate was shown to be a more potent PTPs inhibitor than Na<sub>3</sub>VO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> [50]. Peroxovanadium complexes appear to be 100–1000 times more effective inhibitors of PTPs in vitro than sodium orthovanadate [46,47].

The oxidation state of vanadium may also play a role in the activation of intracellular signal transduction pathways regulating the activation of cytosolic protein tyrosine kinases [50,51].

## 2.2. Vanadium biokinetics, distribution and metabolism

The entry of vanadium into the organism depends on the route of administration. Once it has entered, vanadate (V) is reduced to vanadyl (IV) by glutathione of erythrocytes or by ascorbic acid (AA) and other reducing substances in plasma. It is then transported by albumin and by transferrin, one of the iron-containing proteins with which vanadium probably associates selectively [52,53].

At the cellular level, however, vanadate (V) predominates due to neutral pH. Vanadium (V) enters the cell via the anion transport mechanism [54,55]. Then vanadate (V) becomes reduced to vanadyl (IV) mostly by the intracellular glutathione [4,36] and is subsequently bound to proteins. This reduction is regulated by the cellular redox mechanisms which control the equilibria between the vanadium oxidation states [3,4,56]. Studies on erythrocytes indicate a two-phase mechanism of vanadium entrance. During initial phase vanadate (V) equilibrates across the red cell membrane entering the cell via the anion exchange system. The second, much slower phase, depends on the reduction of V(V) to V(IV) [3,4]; entry of vanadium (V) in tunicate blood cells of *Ascidia nigra*, via the anionic channel, has revealed that vanadium (V) is stored in cell vacuoles, and is then reduced to V(IV) and/or V(III) both of which, being cationic, do not permit vanadium to escape from vacuoles [57].

Vanadium (V)-48 uptake by bovine kidney cells (MDBK) revealed that it is accumulated in cells in linearly between concentrations 20 and 200  $\mu\text{M}$   $\text{Na}_3\text{VO}_4$  and proportionally to its concentration in the medium. A curvilinear relationship between the cellular vanadium content and the degree of vanadium-induced cytotoxicity was also shown [3].

Vanadium in cells is mainly distributed in the nucleus and the soluble supernatant fractions [3,56,58–60]. There is also evidence that vanadyl (IV) and vanadate in the cytosol is bound to selected phosphate, carboxyl and amino ligands [35,61]. Neckay et al. [61] calculated that in cells with phosphocreatine (CP), 61% of total V(IV) is bound to phosphates and 29% to proteins. In cells with high levels of AA, such as the brain cells, ascorbic binds up to 3% of V(IV). The rest is probably bound to carboxyl ligands and to sulfhydryl compounds, whereas almost 1% remains unbound. Bracken et al. [3] reported that vanadium in MDBK cells is distributed at almost 90% in the supernatant fraction; the rest, is mainly in the mitochondria, the nuclear and cell debris, and the microsomes. In a more recent study vanadium administered in rats at tetravalent and pentavalent oxidation state was intracellularly distributed mainly in the nuclear fraction (30–40%), followed by cytosol and mitochondrial fractions. Gel filtration chromatography of the lung cytosol showed, also, two biochemical pools of vanadium; one corresponding to the protein-bound vanadium and another to the diffusible vanadium forms [60]. Administration of vanadocene dichloride to cancer-bearing mice (transplanted by Ehrlich ascites tumor) and Ehrlich ascites tumor cells revealed that vanadium, estimated by electron energy loss spectroscopy was mainly accumulated in the nuclear heterochromatin and to a minor extent, in the nucleus and the cytoplasmic ribosomes of the tumor cells [25].

Various studies in animal models indicated that vanadium mainly accumulates in kidneys, spleen, bones, liver and to a lesser extent in lungs and testes as vanadyl (IV) [4,60,62,63] with bones being probably the main tissue of vanadium accumulation [10,64–66]. Vanadium is rapidly excreted by the kidneys with a biological half-life of 20–40 h in urine [2].

There is also some evidence that vanadium accumulates more in cancer cells and tissues than in normal ones. The levels of vanadium, estimated by X-ray energy fluorescence, were found to be significantly elevated in cancerous breast tissue in comparison to normal breast tissue [67]. Inorganic  $^{48}\text{V}$ , was highly accumulated in MH134, S180 and FM3A tumors, though to a lesser degree than a  $^{48}\text{vanadyl-pheo-phorbide complex}$  [68]. There is also evidence that vanadium administered in tumor cell lines such as leukemias (P388 and L1120), colon, Lewis lung carcinoma, melanoma (B16), solid and fluid Ehrlich ascites tumors

and lung carcinomas transplanted into athymic mice, accumulates in nucleic acid-rich regions of tumor cells [21].

### 2.3. Toxicity of vanadium compounds in humans and animals

Administration of vanadium compounds in humans and animals revealed that vanadium may exert various toxic effects. Studies on animals (mainly rats and mice) showed that the toxic effects of vanadium compounds are related to the species, the dose, the route and the duration of administration as well as to the nature of the compound. Studies dealing with the administration of vanadium salts in humans, suffering from either diabetes mellitus or coronary artery disease indicated that with short-term treatment (maximum for 4 weeks) the most common side-effect was mild gastrointestinal disturbance [69–71]. Long-term treatment (for about five months, with 125 mg/day of a vanadium salt), induced in almost half of the subjects treated (coronary artery disease patients), anorexia, weight loss and abdominal pain [72]. Fawcett et al. [73] pointed out that oral administration of vanadyl sulfate in humans has no effect on blood cells, viscosity and biochemistry.

Studies on animals indicated a variety of toxic effects induced by vanadium compounds. Functional disturbances and histopathological alterations of liver and kidneys are the most common toxic effects. Oral administration of  $\text{NaVO}_3$  (between 5 and 10  $\mu\text{M}$ , in the drinking water) for 3 months, induced mild and dose-dependent histopathological lesions in kidneys and spleen accompanied by increased plasma concentrations of urea and uric acid at the highest exposed groups [74]. Vanadium-induced chromosomal aberrations and aneuploidy-related endpoints in bone marrow of mice following intragastric treatment with vanadyl sulfate, sodium orthovanadate and ammonium sulfate, have also been reported [75]. Studies in young and adult rats receiving intraperitoneal injections of orthovanadate (10 mg/kg/day for 8 consecutive days), revealed that vanadium may cause nephrotoxicity, its severity being related to the age of animals treated [75]. Significant reductions in general activity and learning have also been shown in healthy rats administered orally with vanadium at doses between 4.1 and 16.4 mg/kg/day for 8 weeks [76].

Toxic effects on the reproductive and developmental functions of rats and mice such as a negative impact on the rate of conception and fetal development, by vanadium compounds, have also been well-established [77,78]. Studies however, on healthy and diabetic rats indicated no significant changes in hematological parameters, hepatic and renal function or characteristic morphological abnormalities in various organs [79,80]. We recently showed that per os administration of

vanadyl sulfate and V(III)–cysteine complex in male Wistar rats for 9 weeks, at daily vanadium doses of 14 mg/kg, did not induce any hematological, biochemical or histopathological alterations. V(III)–cysteine-treated rats exhibited a slight weight gain, in contrast to vanadyl sulfate-treated rats, which exhibited a slight weight loss, but both were not significantly different than their age- and sex-matched controls [42]. Furthermore, long-term (between 4 and 6 months), oral administration of vanadium (III and IV) at daily V doses of 0.5 mg/kg/day, induced no toxic effects, in tumor-bearing rats [40,41].

Acute toxicity of vanadium compounds in animals includes weakness, loss of appetite, dehydration, significant reduction of body weight, nose bleeding, pulmonary hemorrhage, necrosis of lymphoid tissues, renal tubular necrosis and death. Such effects are time and dose-dependent in subcutaneous or intraperitoneal administration of vanadium compounds [39,81]. The nature of the vanadium compound may also be related to the acute toxic effects of vanadium, with V(V) being, in general, more toxic than V(IV) [82]. Vanadium oxides may also be more toxic than vanadium salts. Genotoxic damage in six different organs (liver, spleen, heart and bone marrow) have, for example, been reported in male mice treated by intraperitoneal injections of vanadium pentoxide ( $V_2O_5$ ) (at doses between 5.75 and 23.0  $\mu\text{g/g}$ ) [83]. Toxicity of peroxo heteroligand vanadates (V) in mice was reduced by chelation of heteroligands and toxicity of vanadium salts were also different among salt speciation [14,84]. Short- and long-term side effects of vanadium compounds are also related to the rate of accumulation of the metal in certain organs and tissues of the exposed organism [5].

In conclusion, vanadium compounds do not exert significant toxic effects when administered per os, the duration of administration preserves significant accumulation into target organs and the total and daily dose (per kg weight) is under the toxic limits of the compound used. The majority of the studies conducted on various types of cells indicated only a potential carcinogenicity of vanadium salts [36,85–88]. Potential mutagenic effects of vanadium are also related to the nature of the vanadium compounds and vanadium-derived reactive oxygen species [89,90]. Cancer induction by vanadium compounds in experimental animals has so far being unsuccessful.

### 3. Anticancer effects of vanadium compounds on experimental carcinogenesis and tumor-bearing animals

Chemopreventive and antitumor effects of vanadium compounds have been widely investigated, on experimental animal models and various types of malignant cell lines.

The first evidence that vanadium may exert chemopreventive effects on experimental carcinogenesis, was provided by Thompson et al. [9] on MNU-1 (1-methyl-1-nitrosourea)-induced mammary carcinogenesis in female Sprague–Dewley rats. Feeding the animals with 25  $\mu\text{M}$  per day vanadyl sulfate (IV) resulted in a reduction of both cancer incidence and the average number of cancers per se, and prolongation of the median cancer-free time. Further studies on DMBA (7,12-dimethyl-benz( $\alpha$ )-anthracene)-induced mammary carcinogenesis in female Sprague–Dewley rats, revealed that supplementation with ammonium monovanadate (0.5  $\mu\text{M}$ ) in the drinking water, administered ad libitum to the rats, resulted in a significant reduction of incidence, total number, multiplicity and size of palpable tumors and delay in mean latency period of tumor appearance. Chemopreventive effects of vanadium on DMBA-induced mammary carcinogenesis were mediated through alteration on hepatic antioxidant status of animals, as well as modulation of phase I and phase II drug metabolizing enzymes [91]. The chemopreventive effects of dietary (0.5  $\mu\text{M}$ ) ammonium metavanadate were further shown against DEN (diethylnitrosamine)-induced and promoted by phenobarbital hepatocarcinogenesis in male and female Sprague–Dawley rats [91–93]. The chemopreventive action of vanadium was found to be mediated through inhibition of altered liver cell foci and hepatic nodule growth during the early stages of neoplastic transformation. Reversal of liver cell foci and hepatic nodule growth by vanadium supplementation, was also related to the activity of  $\gamma$ -glutamyl transpeptidase and to the restoration of decreased hematological parameters (hematocrit, hemoglobin, red blood cell count). Normalization of the depleted plasma albumin concentration and the decreased albumin to globulin ratio of animals were also related to the substantial protection against DEN-induced carcinogenesis by vanadium compounds. Vanadium was also shown to exert more potent antitumor potential on the initiation rather than the promotion stage of hepatocarcinogenesis. Further studies on the effects of vanadium supplementation (0.5  $\mu\text{M}$ ) against DEN-induced hepatocarcinogenesis in Sprague–Dawley rats indicate that chemopreventive effects are probably mediated through an elevation of phase II conjugating enzymes, which in turn lead to a move and shift of hepatic metabolic profile that reduces the intracellular concentration of carcinogen-derived reactive intermediates [94]. Finally, concomitant administration of vanadium with the active metabolite of vitamin D(3) on DEN-induced hepatocarcinogenesis in Sprague–Dawley rats indicate that the chemopreventive effect of the above combination is probably due to the significant protective action on the genetic damage in liver cells upon alkylation induced by DEN [95].

Antitumor effects of vanadium compounds were also shown on male albino mice bearing transplantable Dalton's ascitic lymphoma (DL). Ammonium metavanadate, administered at low doses, prolonged the survival of DL-bearing mice, increasing the levels of various detoxifying hepatic enzymes (glutathione-*S*-transferase and UDP-glucuronyl transferase) and the cytochrome P450 content [96].

Vanadium (ammonium metavanadate 0.005  $\mu\text{g}/0.1$  ml/mouse/day) caused also, significant reduction of the tumor cell proliferation in DL-bearing albino mice through a significant increase (4-fold) of the erythropoietin, which improved the hemato-logical aspects of the treated hosts, and may played a vital role in regulating the growth of cellular neoplasia [97]. There is, however, evidence that ammonium metavanadate at higher doses (0.01–0.1  $\mu\text{g}/\text{mouse}/\text{day}$ , throughout the study) may result in a significant dose-related increase in the tumor cell count and decrease in the survival of DL-bearing albino mice, followed by a sharp increase in the activity of the hepatic glutathione-*S*-transferase and by a shift to the left of the peak of P450 content in vanadium treated mice [98].

Orthovanadate, administered subcutaneously (500  $\mu\text{g}/\text{mouse}/\text{day}$  for 9 days) resulted in a significant reduction (up to 85–100%) of MDAY-D2 tumor growth in mice which was probably due to the generation by vanadate (V), of hydroxyl radicals and other active oxygen species [99]. Various peroxovanadates (V) exerted significant anticancer effects on tumor-bearing mice, their activity being dependent upon the type of heteroligand [13,14].

Our recent results indicated also that vanadyl sulfate (IV) and the organic complex of vanadium (???) with cysteine, administered orally at low vanadium doses (0.5 mg/kg/day) exerted both significant therapeutic effects on leiomyosarcoma-bearing Wistar rats with vanadium (III)–cysteine complex being, however, more potent and less toxic than vanadyl sulfate (IV) [40–42]. However, Kingsworth et al. [100] reported that supplementation in diet or drinking water with vanadate (V), had little or no effect on 1,2-dimethylhydrazine-induced colon cancer in mice. Vanadocenes, mainly vanadocene dichloride, were shown to be potent antitumor agents against intraperitoneally growing mouse mammary tumor (TA3Ha) [7]. Administration of vanadocene dichloride (intraperitoneally, in doses 100–600 mg/kg) in Ehrlich ascites tumor-bearing CF1 mice revealed that the cytostatic activity of vanadocenes was due to the molecular interaction of vanadium with nucleic acids, especially with DNA [25]. Furthermore, the antitumor effects of vanadocene dichloride against human colon and lung carcinomas transplanted in athymic mice, were shown to be due to the vanadium accumulation in nucleic

acid-rich regions and to the inhibition of DNA and RNA synthesis in tumor cells [21].

Collectively, these experimental studies indicated that vanadium compounds exert: (a) chemopreventive effects against chemically-induced carcinogenesis, mainly through inactivation of carcinogen-derived active metabolites, via modulation of the content and the activity of various liver xenobiotic metabolizing enzymes and substrates (P450) and/or increase of the antioxidant status of the carcinogens' target organs; they act mainly in phase I (initiation) and secondly phase II (promotion) of chemical carcinogenesis; (b) antitumor effects on tumor-bearing animals, either through the vanadium accumulation in nucleic acid-rich regions, inhibiting thus DNA and RNA synthesis and/or inducing the generation of cytotoxic reactive oxygen species in tumor cells. There is also indication that vanadium compounds may, in addition, exert specific systemic effects favoring survival and/or regulating tumor growth on cancer-bearing animals. Extensive investigation, on normal and malignant cell lines have, in addition, provided detailed information on the different ways through which vanadium compounds may exert their anticarcinogenic effects (Fig. 1).

#### 4. Cellular mechanisms of the anticancer effects of vanadium compounds

The ideal anticancer treatment by any compound should fulfill certain criteria and exert the following selective effects on malignant cells: (a) reduction of cellular growth rate (antiproliferative effect), (b) cytotoxic and/or cytostatic actions expressed either by necrotic or programmed cell death (apoptosis), (c) reduction and/or inhibition of the invasive or metastatic potential of the cells, and (d) reduced or absent potency to induce cellular resistance. Vanadium compounds possess all of these properties, exerted either in concert or independently, through a variety of cellular mechanisms that will be briefly reviewed.

Anticancer effects and mechanisms exerted by vanadium compounds have been investigated on a variety of malignant cell lines, among which human B cell lymphoma and T cell leukemia [50], murine erythroleukemia [8], rat basophilic leukemia [16], leukemic cells L1210 [13,14], HL-60 and M07e [101], human and rat hepatoma [16–19,102,103], human ovary carcinoma [104], testicular cancer [22], nasopharyngeal carcinoma [105], larynx carcinoma [106], osteosarcoma [5,107] as well as Ehrlich ascites carcinoma [5,108,109], mouse and rat neuroblastoma [106,110], rat glioma [110,111], mouse epidermal JB6 P<sup>+</sup> [18,112–115], Lewis lung carcinoma [116] and Hela cells [42] are the most common cell lines used.

#### 4.1. Effects of vanadium on cell proliferation

Vanadium complexes have been shown to exert either antiproliferative or, in some cases, proliferative effects on various types of cells.

Peroxo vanadium complexes, for example, have been found to reduce the proliferation rate of neuroblastoma NB41 and glioma C6 cell lines [110], whereas, in C3H10T1 mouse fibroblasts, peroxovanadates formed in situ lead to proliferation [117]. Biphasic effects of vanadium compounds (ammonium metavanadate, vanadyl sulfate trihydrate and ortho sodium vanadate) have also been reported on in vitro tumor colony growth. Vanadium salts at low ( $< 10^{-10}$  M) concentrations stimulated, and at higher ( $> 10^{-10}$  M) concentrations inhibited colony formation in human tumors [118].

Antiproliferative effects of vanadium compounds on normal and malignant cell lines appear to be exerted mainly through cell cycle arrest. Fluorescence-activated cell sorting assessments revealed that peroxovanadates block, reversibly at low ( $\mu$ M) concentrations and irreversibly at higher, the  $G_2$ -M transition of the cell cycle

in cancer cells, leading to a significant reduction in growth rate [110]. Cell cycle arrest induced by vanadium complexes appears to be mediated through the inhibition of PTPs, that in turn dephosphorylate subunits of the cyclin-B complex. Phosphorylation of the p34<sup>cdc2</sup> subunit of the p34<sup>cdc2</sup>-cyclin complex is essential for the progression of mitosis [15]. Cell cycle arrest by vanadium complexes may also be exerted through activation of mitogen activated protein kinases (MAPKs superfamily) signaling pathway. V(IV) activates the p38 MAPK and induces the transcription of nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B), a factor involved in both cell-cycle progression and apoptosis, in human bronchial epithelial cells [119].

Vanadium compounds may also exert antiproliferative and cytotoxic effects via interactions with DNA. Vanadocene complexes interact with DNA's nucleotide phosphate groups forming a labile outer sphere complex via a water group. The character of the vanadocene dichloride interaction with DNA is thus different than that of cisplatin which forms covalent DNA adducts that are potentially mutagenic [7,19,120]. Metallocene dichlorides, among which is vanadocene

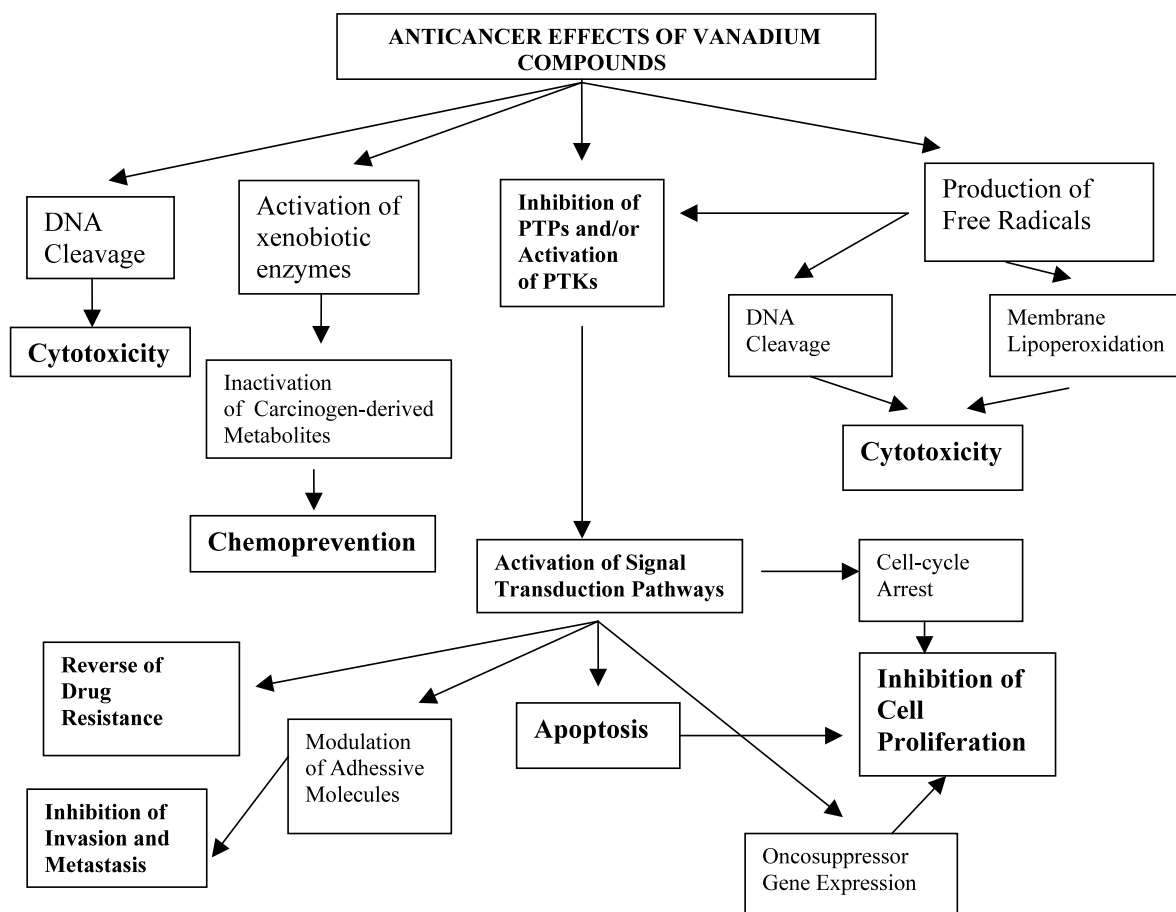


Fig. 1. Schematic presentation of the actions, through which the antitumor effects of vanadium are exerted (PTPs, Protein Tyrosine Phosphatases, PTKs, Protein Tyrosine Kinases).

dichloride, cause alterations of nucleic acid metabolism [24] and form vanadocene–DNA complexes, as shown by ultrastructural studies [21,121–123]. DNA cleavage *in vivo* and *in vitro* is also induced by a variety of vanadium compounds [66,83,105,124–127]. Several lines of evidence indicate that V(IV) and V(V) induce DNA cleavage not directly reacting with DNA components but acting mainly through the production of highly reactive oxygen species, especially hydroxyl radicals (OH<sup>•</sup>) generated in cells. Incubation of DNA with vanadyl ions and hydrogen peroxide led to intense DNA cleavage caused mainly by OH<sup>•</sup> generated during the reactions of vanadyl ion and H<sub>2</sub>O<sub>2</sub> as demonstrated by ESR spin trapping [66]. Salmon sperm DNA exposed to H<sub>2</sub>O<sub>2</sub> and vanadium (III) caused single- and double-strand breaks, mediated by Fenton reactions. The generation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) exhibited, also, a good correlation with the formation of double-strand breaks, suggesting that they may arise by a similar mechanism [127]. Fenton reactions are also involved in the generation of putative intrastrand links and strand breaks by vanadium (III) and other transition metals, as detected by agarose gel electrophoresis of salmon sperm DNA [126]. A newly synthesized vanadyl–1,10-phenanthroline complex [VO(phen)<sub>2</sub>], which inhibits the growth of human nasopharyngeal carcinoma KB cells, was found to cleave supercoiled plasmid Col E1 DNA effectively when hydrogen peroxide was added. ESR spin trapping demonstrated that the active species for DNA cleavage were hydroxyl radicals generated in the VO(phen)<sub>2</sub>–H<sub>2</sub>O<sub>2</sub> system [105]. Vanadyl (IV) induced direct oxidative DNA damage as shown by the redox indicator methylene blue, in human Chang liver cells, degrading both 2N and 4N DNA phases in G1, S and G2/M cell cycle profiles, as revealed by flow cytometric evaluation of cell cycle phase-specific DNA composition [124].

Direct reactions by some oxides of vanadium with DNA should not, however, be excluded. Studies with single-cell gel electrophoresis assay (Comet Assay) and DNA migration, revealed that V<sub>2</sub>O<sub>5</sub> induces DNA single-strand breaks in whole blood leukocytes and human lymphocyte cultures [125] and in testicular cells, in a clear dose–response [83].

#### 4.2. Apoptotic effects of vanadium compounds

Necrosis and apoptosis lead to cell death by two morphologically distinct ways. Necrosis is a result of cytotoxic effect inducing cell swelling, without morphological nuclear changes and cell death due to cellular membrane damage and leakage of cytoplasmic and nuclear contents. Apoptosis, also known as programmed cell death, is mainly characterized by nuclear and cytoplasmic condensation, nuclear fragmentation and membrane blebbing, as well as by the appearance

of small spherical, cytosolic fragments containing pyknotic remnants of nuclei, which are called apoptotic bodies; it is implicated in a variety of physiological processes, but inappropriate regulation of apoptosis may play an important role in many pathological conditions among which is cancer [128,129]. There is a variety of extracellular and intracellular stimuli which regulate cellular signaling pathways, transducing events to the nucleus and inducing cell apoptosis. Effects on cell proliferation rate and cell differentiation, exerted by vanadium compounds may, in contrast to apoptosis, not always be mediated by intracellular signaling pathways. Regulation of certain intracellular signaling pathways by vanadium, may influence a number of cellular functions among which are cellular growth rate, differentiation, transformation, decrease in cell adhesion and programmed cell death.

Accumulating evidence indicate that vanadium compounds modulate the extend and duration of phosphorylation of a number of proteins, such as AP-1, MEK-1, ERK-1, JNK-1, PI-3K and NF-κB [15,112,115,130,131], key effector proteins of the signaling pathways linked to the production of reactive oxygen species and DNA damage. Activation of cell signaling pathways is mediated through the phosphorylation of tyrosines, dependent on the activity of cytosolic protein tyrosine phosphatases and phosphokinases that regulate phosphorylation and dephosphorylation of proteins critical for signal transduction.

Both, inactivation of phosphatases and/or activation of phosphokinases, lead to accumulation of phosphorylated protein tyrosine residues in several cells, the generation of second messengers, the activation of down-stream kinases, and the activation of various factors among which NF-κB is the most important. Activation of NF-κB is preceded by the phosphorylation of its inhibitory subunit (IκB) on two N-terminal serine residues. The IκB masks, in the cytoplasm of most cells, the translocation sequence of the transcription factor of the DNA-binding dimer (NF-κB). Phosphorylation of IκB results in its proteolytic degradation and release of NF-κB dimer, which translocates into the nucleus, binds to NF-κB-specific response elements, and modulates transcription. NF-κB regulates the expression of a variety of genes whose products mediate immune inflammatory responses and regulate cell-cycle progression leading either to the protection from cellular apoptosis or to cellular death by apoptosis [132,133]. It, thus, seems that the anticancer effects of vanadium compounds, in relation to protein tyrosine phosphorylation and to NF-κB activation, depend on whether lethal or apoptotic target genes are activated [128]. The latter seems to depend on either the type of cell activated or the vanadium compound and dose administered, or both. In lymphocytes, for example, in which vanadium compounds induce NF-κB nuclear



translocation, bis(maltolato)-oxovanadium (IV), induces apoptosis in B cell lineages, but enhances the activation of T cell lineages [134]. Four vanadium compounds, vanadate (V), vanadyl (VI), bis(maltolato)oxovanadium (IV) and bis(maltolato)dioxovanadium (V) [BMV], all being promoters of the phosphorylation of tyrosine residues in several proteins of MC3T3E1 osteoblast-like cells, stimulated cell growth at low, but inhibited it at high concentrations, and induced distinct changes in cellular morphology, following overnight incubation. Tyrosine phosphorylation was more pronounced at low than at high doses. BMV, being the least cytotoxic and the weakest inducer of morphological changes, showed, at low concentrations (10  $\mu$ M), a phosphorylation pattern similar to that of insulin [135]. Vanadate also prevented apoptosis in leukemic M07e cells cultured for 24 h in the absence of growth factors [101]. The results suggest that a balance between tyrosine kinases and tyrosine phosphatases establishes whether a cell will survive or undergo apoptosis.

Phosphotyrosine phosphatases (PTPs) serve as important regulators of signal transduction pathways and are sensitive targets of oxidative stress [136,137]. Vanadium induces intracellular inactivation of PTPs [30,45,50], by a variety of biochemical mechanisms depending mainly upon the oxidative state of vanadium (Section 2.1). However, recent evidence on the *in vivo* inhibition of PTPs by vanadium compounds show that they may or may not be related to cellular apoptosis.

Enhancement of tyrosine phosphorylation due to the inhibition of membrane associated tyrosine phosphatases by pervanadate was reported in prostatic epithelial cells [138] and vanadate selectively inhibited the protein tyrosine phosphatases of intact rat hepatoma cells [17]. Orthovanadate, at concentration of 1–10  $\mu$ M, effectively inhibited PTPs in the BALB/3T3 cells, promoting 3-methylchloranthene-pretreated cell transformation [139]. Various vanadium compounds (IV and V) were shown to inhibit alkaline phosphatase activity in osteosarcoma UMR 106 cells and osteoblasts [107]. There is also substantial evidence that inorganic salts of vanadium activate phosphotyrosine phosphorylases, specifically the MAPKs [18,112,119,140–142]. MAPKs, which include extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SARK) and p38 subfamilies, are important regulatory proteins through which various extracellular signals are transduced into intracellular events. They are responsible for the phosphorylation of a variety of proteins including downstream kinases and transcription factors that are activated by a variety of stimuli and different cellular stresses, mainly by the oxidative stress. Both the ERKs and the JNK/SARK signaling pathways have also been implicated in NF- $\kappa$ B activation through phosphorylation of its inhibitor I $\kappa$ B

[143]. Phosphorylation of I $\kappa$ B is mainly mediated by a large multisubunit I $\kappa$ B kinase complex (IKK) that is activated by an upstream kinase, the NF- $\kappa$ B-inducing kinase which in turn, is a mitogen activated protein kinase (MAPK) kinase kinase (MAPKKK) [144]. JNK and ERK kinases may, however, have opposite effects on apoptosis [145].

There is a number of recent data indicating that vanadium compounds are implicated in NF- $\kappa$ B activation in normal and cancer cells through activation of MAPKs signal transduction pathway, leading to cell apoptosis [131,141,146]. Vanadium compounds activated the MAPKs family in epithelial cells of the ovaries [112,131] and the lungs, specifically the p38 [119], resulting in the activation of NF- $\kappa$ B, in the cytoplasm. Vanadium (IV) induced I $\kappa$ B breakdown in human airway epithelial cells and NF- $\kappa$ B nuclear translocation [142]. Jaspers et al. [119] also provided evidence that the activation of p38 MAPK by vanadyl sulfate in human airway epithelial cells depends on V(IV)-induced oxidative stress and mediates  $\kappa$ B-dependent transcription without affecting nuclear translocation or DNA binding of NF- $\kappa$ B, demonstrating that V(IV)-induced oxidative stress activates collateral signaling pathways which converge downstream to cooperatively modulate the transcriptional activity of NF- $\kappa$ B. Bis-peroxovanadium (bpV), a potent PTPs inhibitor, activates NF- $\kappa$ B in human T lymphocytes engaging the src family protein kinase p56<sup>lck</sup> and downstream effectors of p56<sup>lck</sup> such as the syk family protein kinase ZAP-70 and the molecular adaptor SLP-76 [147].

The *in vivo* effects of vanadocenes to suppress tumor cell growth and reduce hyperlipidemia and hypertension via the tyrosine kinase signaling pathways have also been well documented [7,10,12,22]

There are also other signaling pathways activated by vanadium which may lead to apoptosis. Production of reactive oxygen species by vanadium compounds in the cytosol or in the mitochondria, results in mitochondrial damage and the release of cytochrome C which activates the caspases. Activated caspases such as caspase-3 and caspase-8, provoke further mitochondrial damage and activate cellular substrates such as poly(ADP-ribose)-polymerase (PARP), leading the to apoptosis [148,149].

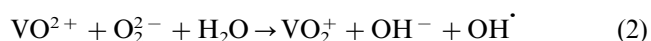
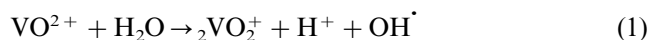
Recent studies have shown that administration of vanadium salts to mouse epidermal cells induced apoptosis through the production of hydrogen peroxide and other ROS which in turn, activated p53 [113], a tumor suppressor gene involved in cell cycle regulation, DNA repair and apoptosis [150]. Cytotoxic vanadocene derivatives induced, however, apoptosis in human cancer cells, by triggering the apoptotic signal not by primary DNA-damage or requiring p53 induction disproving the hypothesis that they mechanistically resemble the

cytotoxic action of *cisplatin* [19]. Five metallocene dichlorides, among which is vanadocene dichloride, induced apoptosis in human testicular cancer lines [23]. Fifteen newly synthesized oxovanadium (IV) compounds, examined for their cytotoxic activity against 14 different human cancer cell lines induced apoptosis, especially the leading compound  $\text{VO}(\text{Me}_2\text{-phen})_2$  [151].

It is, thus, evident from research data, that vanadium compounds activate in normal and cancer cells, different signaling pathways acting mainly, through inactivation of PTPs and/or activation of PTKs. Activation of cellular signaling pathways seems mainly to converge into NF- $\kappa$ B nuclear translocation and the transcription of either apoptotic and lethal or antiapoptotic genes. Activation of apoptosis through mitochondrial caspases should not be overlooked. Research going on will, probably soon, provide substantial evidence for the chemical properties and biochemical effects of vanadium compounds as well as the various types of cancer cells on which vanadium, acting intracellularly, may exert selective apoptotic and/or lethal effects.

#### 4.3. Vanadium-induced cellular oxidative stress

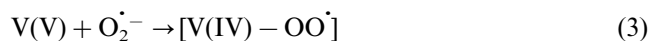
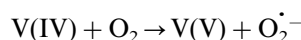
Vanadium, possessing as a transition metal various oxidative states, may participate in free radical generating reactions [152]. In plasma, vanadium (V) is rapidly reduced to V(IV) mainly by plasma antioxidants and is transported bound to plasma proteins, whereas pentavalent vanadium usually predominates in extracellular space (see above). The theoretical possibility of extracellular free radical generation by V(IV)/V(V) species, mainly found in biological fluids, should, however, not be overlooked. Intracellularly, vanadium is mainly at tetravalent oxidation states, and it is also bound to cytosolic and nuclear fragments, but a small proportion (almost 1%) may be found in an unbound form. Moreover, in cancer cells, differing from normal in a number of ways including a lower pH, an altered level of antioxidant enzymes, a different redox state, a higher water content and a constant  $\text{H}_2\text{O}_2$  production [153], free radical generation by vanadium may easily take place by a variety of chemical and biochemical reactions. In weakly acid environments, with pH around 7, in the presence of V(IV)/V(V),  $\text{H}_2\text{O}_2$  and oxygen, a free radical production may take place by, mainly, the following chain reactions [7]:



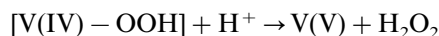
Generation of highly toxic hydroxyl radicals ( $\text{OH}^\cdot$ ) may in turn trigger further radical reactions leading to the relative benign superoxide anion radical, which is spontaneously dismutated by superoxide anion dismutase (SOD), to oxygen and  $\text{H}_2\text{O}_2$ , that, if nothing

interrupts, may, reacting with vanadium ( $\text{VO}^{2+}$ ), perpetuate hydroxyl radical generation. V(IV) may, in vivo, reacting with oxygen to generate superoxide anion and V(V) [154]. Fenton-like reactions may also take place intracellularly, mainly with the unbound vanadium [89,90,126,127]. Reactions as above have been shown by EPR spectroscopy to take place during the reduction of V(V) to V(IV), by three typical NADPH-dependent flavoenzymes, glutathione reductase (GSH-R), lipoyl-dehydrogenase and ferredoxin-NADP<sup>+</sup>-oxireductase, during which generation of hydroxyl radicals take place [37,38]. Superoxide anion radical was not significantly involved in the enzymatic reduction of V(V) to V(IV). The enzymes-vanadium-NADPH mixture caused, however, the reduction of molecular oxygen to generate hydroxyl radicals which in turn was enhanced by exogenous  $\text{H}_2\text{O}_2$ , a phenomenon suggesting the occurrence of a Fenton-like reaction [37,50,89,90].

Peroxo- and hydroperoxide vanadium (IV) radicals can also be formed in cells by the superoxide generated by NADPH-oxidase. NADPH, the substrate of the respiratory burst oxidase is required for the synthesis of superoxide anion in various cells. Superoxide in turn triggers the formation of peroxovanadyl [ $\text{V}(\text{IV}) - \text{OO}^\cdot$ ] and vanadyl hydroperoxide [ $\text{V}(\text{IV}) - \text{OH}^\cdot$ ]. Vanadium peroxides can also be formed by the following reactions, mediated by NADPH [155]:



Reactions as above demand also NAD(P)H-dependent flavoenzymes [37]. Peroxovanadyl (reaction 3; Eq. (3)), can in turn remove hydrogen from NADPH, and produce vanadyl hydroperoxide. The latter in the presence of  $\text{H}^+$  can decompose producing  $\text{H}_2\text{O}_2$  and regenerate vanadate as follows:



These peroxides promote the inhibition of phosphotyrosines, resulting in accumulation of tyrosine phosphorylated proteins (TPPs). Critical accumulation of TPPs initiate a respiratory burst with abundant production of superoxide which then catalyzes the formation of additional vanadium (IV) peroxides, establishing a vicious cycle [156].

Various studies on the mechanisms of the actions of vanadium compounds indicate also that vanadium induces oxidative stress by a number of different ways.

Vanadium compounds may, for example, indirectly modify oxidative stress in cells and animals, either releasing free radical generating metals from tissues or modifying enzymatic and antioxidant defense.

There is evidence that V(IV), but not V(V), is able to mobilize in vitro, iron from ferritin, which is a source for oxidative reactions in biological systems. Iron release may be prevented in presence of relative excess to the concentration of vanadyl (IV), of glutathione (GSH) or vanadate. Vanadyl (IV), but not vanadate (V), is in turn promoting peroxidation in phospholipid peroxosomes [154]. Vanadyl (IV), in presence of H<sub>2</sub>O<sub>2</sub>, generates hydroxyl radicals in the vanadyl-catalyzed-break-down of fatty acids and hydroperoxides, which in turn initiate diene conjugation during V(IV)-catalyzed lipoperoxidation [155,157].

Vanadate, administered in diabetic and non-diabetic rats, produced significant decreases in the antioxidant enzyme catalase (CAT) activity and reduced GSH levels in animals liver [158]. In contrast, supplementation of drinking water with vanadium at the level of 0.2 or 0.5 μM for 4–8 weeks resulted in a significant increase of the GSH level with a concomitant elevation of glutathione-S-transferase (GST) activity in the liver, the small and large intestine mucosa and kidney in rats, without any apparent signs of cytotoxicity [159,160]. Carcinoma cells harvested from Swiss albino mice, transplanted intraperitoneally with Ehrlich ascites carcinoma cells, showed, after treatment with vanadium (IV), a significant decrease in glutathione peroxidase (GSH-Px) and GSH-R with concomitant increase in SOD and glucose-6-phosphate dehydrogenase activities. Both tumor volume and cell viability were significantly lowered in vanadium (IV)-treated mice [108].

These in vivo studies, though providing some conflicting results are partly, in contrast to some in vitro studies dealing with the effects of vanadium on the activity of the main antioxidant enzymes, horseradish peroxidase, catalase, GSH-Px and superoxide dismutase. V(IV) and V(V) showed any direct effect on the activity of these main antioxidant enzymes, suggesting that many biological and toxicological effects of the metal may be mediated more by oxidative reactions of vanadium or of its complexes with physiologically relevant biomolecules, than by a direct biochemical modulation of enzymatic activities [161,162].

Accumulating evidence indicate that vanadium-induced oxidative stress and free radical production is strongly implicated for the majority of anticancer, toxic and molecular effects of vanadium compounds such as PTPs inhibition and PTKs activation [7,14,16,45,50,51,108,137,156], MAPKs-dependent signal transduction [114,131,137,141,143,146], NF-κB translocation [112,119], DNA cleavage and strand breaks [65,105,112,129,122,126,127], gene and oncogene expression [81,86,88,113,115] and cytotoxicity [5,31,36–38,152,157,185].

Free radicals produced by vanadium compounds, mainly the reactive oxygen species, may also exert general systemic effects in organisms, through mem-

brane lipoperoxidation, protein denaturation and DNA damage [90].

## 5. Potential antimetastatic effects of vanadium compounds

Protein tyrosine phosphorylation induced by vanadium compounds, may also influence the invasive and metastatic potential of tumor cells, regulating cell-substrate adhesion [116] or cell-to-cell contact and actin cytoskeletal changes [163–165]. Adhesive proteins (cell to cell or cell to matrix) are involved into cancer metastasis and invasion [166,167].

Vanadium compounds may also prevent hematogenous spread of cancer by activating tumor-induced endogenous fibrinolysis and inhibiting platelet aggregation, both involved in hematogenous metastasis of cancer [166,168,169].

Pervanadate has been shown to inhibit the induction of intracellular adhesion proteins ICAM-1, V-CAM-1 and ELAM-1 in endothelial cells. This inhibition was probably exerted through the inactivation of PTPs and the subsequent downmodulation of tumor necrosis factor (TNF) which is one of the major inducers of various adhesion molecules in human endothelial cells. Inhibition of PTPs by pervanadate also blocked the attachment of monocytes to endothelial cells [164]. Moreover, enhancement of tyrosine phosphorylation by vanadate, resulted in the inhibition of cadherin-mediated aggregation (cell-to-cell adhesion) of rat 3Y1 cells transformed with v-src and doubly transformed cells with v-src and v-fos (SR3Y1 and fos-expressing SRY1 cells with enhanced acquired metastatic potential) [163]. Sustained pervanadate treatment of cells expressing mutant E-cadherin derivatives, previously cloned from diffuse-type gastric carcinoma, led to loss of cell-to-cell contact and induced actin cytoskeletal changes was due to tyrosine phosphorylation of components of the cell adhesion complex [165].

Treatment of high metastatic Lewis lung carcinoma A11 cells with sodium orthovanadate resulted in a dose- and time-dependent suppression of cells spreading on various extracellular matrix such as matrigel, fibronectin, laminin and type IV collagen [116] but it did not significantly inhibit the attachment of the cells to these substrates. Suppression of cell spreading, however, was not directly due to the inhibition of cell growth induced by the orthovanadate.

It was also shown that vanadate, acting as phosphatase inhibitor may stimulate the urokinase-type plasminogen activator in LM3 murine mammary tumor cells [170] modulating thus blood coagulation. Furthermore, orthovanadate, may reduce platelet aggregation induced by thrombin receptor activation, preventing the integrin-dependent tyrosine dephosphorylation of

P38 and P140, and the binding of platelets to fibrinogen [171], an effect implicated in platelet aggregation and tumor metastasis [172]. Vanadate is also an inhibitor of collagen-induced platelet aggregation and release reaction in a concentration dependent fashion [173]. Platelet/endothelial cell adhesion molecules' activity seems also to be modulated via inhibition of phosphatase activity induced by orthovanadate [174].

Our recent (unpublished) results indicate that oral administration of a newly tested vanadium (III)–cysteine complex, at low daily vanadium doses (0.5 mg/kg b.w.), in tumor (leiomyosarcoma)-bearing rats, results in a significant inhibition of lung metastases (up to 80%) in comparison to the control and to vanadyl sulfate (IV)-treated group, receiving orally the same low vanadium doses.

## 6. Drug resistance and vanadium compounds

P-glycoprotein (P-gp), a member of the ABC family of transport proteins, confers multiple drug resistance by acting as a plasma membrane localized ATP-dependent drug efflux pump, in tumor cells. P-gp uses energy from ATP hydrolysis to efflux the cytotoxic compounds against a concentration energy [175,176]. Altered cellular accumulation of antitumor agents is thus considered as a major factor in acquired and intrinsic resistance of tumor cells to chemotherapy.

Resistance to vanadium has been found in certain microorganisms (such as *saccharomyces* and *candidas*) being either mutants manifesting alterations in protein phosphorylation growth control or manifesting defective plasma membrane ATPase [177,178]. There is however evidence that vanadium compounds may sensitize multidrug resistant cancer cells to the cytotoxic effects of various antineoplastic agents inhibiting the ATPase activity. Inhibition of ATPase reduces drug efflux by P-gp, probably decreasing the binding capacity of the transported substrate [176,179,180]. Further, vanadate may destroy the actin fibers of the cytoskeleton of multidrug resistant cancer cells being thus cytotoxic and possibly beneficial as an adjuvant in the chemotherapy of solid tumors [181]. There is, in addition, data indicating that sodium orthovanadate reverses the multidrug resistance of CEM/VLB 100 cells to vinblastin (VLB) not by altering VLB efflux but probably stimulating its influx [182]. Activation of certain protein kinases by some vanadium compounds, such as vanadate may, in contrast, increase the resistance to vinblastine, in multidrug resistant cells, increasing the phosphorylation of the multidrug transporter P-gp in plasma membrane vesicles, probably by a GTP-regulated phosphorylation [183]. Moreover, vanadium in the presence of chelating agents sensitized *cis*platin-resistant human ovarian cancer cells to the cytotoxic effects of *cis*platin [184].

Resistance of murine leukemia and human colon adenocarcinoma cells to the cytotoxic activity of FCE 245517 (tallimustine) was also, reduced by vanadium compounds and other phosphatase inhibitors and decreased by protein kinase inhibitors, suggesting that the activity of some chemotherapeutics are strictly dependent on the presence of tyrosine phosphorylated proteins [185]. Inhibition of cellular phosphatases activity by sodium orthovanadate has, however, been shown to protect tumor cells from the cytotoxic effects of TNF [186].

It is thus obvious that the effects of vanadium on multidrug resistant cells, in relation to cancer chemotherapy, depends upon the type of cell and the antineoplastic agent investigated, as well as on whether inhibition or enhancement of phosphorylation of certain proteins, predominates. The latter is partly related to the chemical and biochemical characteristics of vanadium compounds.

## 7. Conclusions—perspectives

Research proceeded during the recent decades, enriched our knowledge on the chemical and biochemical properties, as well as the mechanisms of systemic, cellular and molecular antitumor effects of vanadium compounds. The anticarcinogenic effects of vanadium, in combination to its low toxicity, established also, by its administration in humans, suggest vanadium as a candidate antineoplastic agent against human cancer. New complexes being more potent and less toxic favor this perspective. However, a vast array of questions seeking immediate answers, demand more intensive basic and applied research in order vanadium compounds to be established as a new class non-platinum group, effective antitumor agents.

## Reviewers

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## Biography

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