Chapter 8
Vanadium in Cancer Prevention

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Abstract The pharmacological role of vanadium in health and disease remains one of the fascinating stories in biology. Recent studies have established vanadium as a novel regulator in assessing physiological and biochemical states of the animals. Vanadium exhibits biphasic effect, essentiality at low concentrations (0.05 μM) and toxicity at high doses (>10 μM). Vanadium inhibits growth of transformed cancer cells in culture. Various laboratories have confirmed the antitumorigenic potential of vanadium in liver, breast and colon cancer in vivo and various human cancer epithelial cell lines in vitro. Antiproliferative and induction of apoptosis may be the major mechanism of vanadium mediated inhibitions of cancer. Vanadium can play a central role in modulating phosphorylation states of various proteins in the cell and can affect many cellular processes regulated by cyclic AMP. In human vanadium is of interest pharmacologically but confirmation to its essentiality will require more significant information from experimental, clinical and epidemiological studies.

Keywords Vanadium • Cancer • DNA damage • Antiproliferative • Key regulator

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8.1 Introduction

In present times, cancer is considered as one of the most fatal threats against civilization. At the late period of nineteenth century, a venture was pioneered by Dr. Stephen Paget in order to understand the underlying mechanism of metastasis [1] so as to develop a therapeutic to be used against cancer. From that very date, researchers and scientists have been engaged in enriching our machineries against cancer. Studies undertaken in the last three decades suggest that the dietary micronutrient vanadium could be a promising arsenal against cancer. At the beginning of nineteenth century the soft, ductile, silver-grey, nonplatinum group transition metal was introduced to us by Andres Del Rio and Nils Sefstorm separately [2]. Almost after 200 years of its discovery, it was found that various marine species have this metal as essential micronutrient [3]. This fact influenced nutritionists to consider vanadium as a dietary micronutrient. Vanadium has been documented in the list of 40 essential micronutrients and is required in trace amount for normal metabolism, proper growth and development of mammals [4]. Vanadium deficiency in nutrition results in growth inhibition, metabolism of thyroid and bones, disorders of generative function, disturbances of lipids and carbohydrate metabolic pathways [3–5]. Increased abortion and peritoneal death rates, growth impairment of tooth, bone and cartilage, decreased milk production during lactation [6], hepatic lipid and phospholipid changes [7], nutritional oedema [8] are usual physiological consequences of vanadium deficiency. Various dietary sources have been considered as the major source of exposure to vanadium for human population though it is present in very low concentration in diets (<1 ng/g) [9]. Vegetables like mushroom, dill seed, parsley, black pepper and foods like cereals, fresh fruits, shellfish are the common dietary sources enriched in vanadium [9–11]. Its intracellular concentration is approximately 20–200 nM. Vanadium is mostly accumulated in bone, kidney, spleen, liver, lung and blood [12]. Though this widely distributed but low abundant element is available in six oxidation states (-1, 0, $+2$, $+3$, $+4$ and $+5$); only $+4$ (vanadyl; VO^{2+}) and $+5$ (vanadate; $H_2VO_4^-$) are physiologically relevant [13, 14]. The pharmacological potentiality of this dietary micronutrient was initially exploited in the treatment of diabetes. Most of the vanadium salts like sodium metavanadate, sodium orthovanadate, vanadyl sulphate are orally administered to the individuals in insulin therapy which give them advantages over their predecessors [15]. Among the other pharmacological activities diuretic action, antiobesity, antihypertension, antihyperlipidemia, enhancement of oxygen affinity are needed to be mentioned [2]. Almost 50 years earlier the first attempt to exploit the pharmacological potentiality of vanadium in anticancer research was made [16]. This was just the opening of an avenue in cancer research. Later on, Thompson and his co-workers studied the chemopreventive effect of vanadium on 1-methyl-1-nitrosourea induced mammary cancer model in rats [17]. Antineoplastic effects of vanadium salts were established against rat liver tumors [18], fluid and solid Ehrlich ascites tumors [19], TA3Ha murine mammary carcinoma [20]. Some peroxovanadates have shown their inhibitory effects against

certain forms of leukemia. But we have to remember that the role of vanadium is not throughout beneficiary in cancer research, it has some detrimental role in the modulation of carcinogenesis. Being accumulated in considerably high amount vanadium may be responsible for haematological and biochemical alteration, renal toxicity, immunotoxicology and reproductive effect. The story of vanadium in the last three decades has shown its positive and negative sides, and thus gives rise to apparent controversies whether the dietary micronutrient could be a promising therapeutic against cancer. The story has its ups and downs and could be named as "The rise and fall of vanadium".

8.2 Physicochemical Properties of Vanadium

Vanadium, chemically classified as transition element, was placed in the fourth period of the VB group in the periodic table. The element is available in six oxidation states, namely −1, 0, +2, +3, +4, +5. Among these oxidation states, vanadic form (+3), vanadyl form (+4) and vanadate form (+5) are the most common [21]. Vanadium mainly occurs in uranium mines and is a constituent of titaniferous magnetites [22, 23]. In fossil fuels such as crude oil, coal, carbonaceous fossils vanadium present as a major trace element [21, 24]. Among the physiologically relevant form of vanadium, +4 (vanadyl) is the most stable form. But in presence of oxidizing agents, i.e. in oxygenated blood vanadium preferably exhibits its +5 oxidation state [25]. Vanadium can inhibit a number of enzymes. There are also a variety of enzymes which are stimulated by vanadium [26, 27]. The enzymes inhibited by vanadium include Na-K-ATPase, H-K-ATPase, phosphoenzyme ion-transport ATPase, myosine ATPase, dynein, adenylate kinase, phosphofructokinase, choline esterase. On the other side, glyceraldehyde-3-phosphate dehydrogenase, lipoprotein lipase, tyrosine phosphorylase, glucose-6-phosphate dehydrogenase, glycogen synthase, adenylate cyclase, cytochrome oxidase [28, 29] are stimulated by its action.

8.3 Pharmacology of Vanadium

At the turn of nineteenth century, some French physicians employed vanadium as a cure-all for ailments such as anaemia, diabetes, tuberculosis and chronic rheumatism [4]. Pharmacological exploitations have since been experiencing a current resurgence in these areas of research. Vanadium as vanadate in the cell has been proven to have specific role in the regulation of many enzymatic activities [30, 31], in generation of free radicals [32, 33], phosphorylation and dephosphorylation of proteins [34, 35]. Vanadium, the well-known prooxidant oxidises NADH and other intracellular reducing agents and generates active reducing form [36]. Tyrosine phosphorylation of a variety of cellular proteins is likely modulated by vanadium

through inhibition of protein tyrosine phosphatases. It also inhibits dephosphorylation of inositol phosphatases [37]. On the basis of its nutritional significance, physicochemical properties, distribution in tissues, it has been considered as a potent physiological regulator of the Na^+-K^+ pump [38, 39]. Vanadium as vanadate can mimic and potentiate the effect of growth factors like insulin [40], epidermal growth factor (EGF) on intact cells [41, 42]. Its insulinomimetic properties and its possible role in the alleviation of diabetic symptoms is one of the most prominent research interests. Vanadium, in vivo has been shown to exert insulin-like effects on transport, glucose oxidation, potassium uptake as well as on the activity of glycogen synthase in an isolated rat adipocytes and skeletal muscle [43]. By regulating the activity of secondary messengers, it can affect signal transduction in the cell. By activating PLC-coupled G-protein, vanadate stimulates consequently IP_3 synthesis in different cells [44, 45]. Increase in IP_3 levels leads to mobilization of Ca^{2+} from intracellular stores and the subsequent increase in intracellular Ca^{2+} level. This phenomenon is important in the stimulation of many cellular processes but excess Ca^{2+} can also be toxic [46]. Activation of adenylate cyclase by vanadium leads to increased cAMP level [47] but inhibition of cAMP production by vanadate and absence of effect on cAMP level were also described [48]. Thus vanadium can modulate many cAMP regulated cellular processes. On carbohydrate metabolism the effect of vanadium can be related to the reduction of the plasmatic glucose concentration. The vanadate ion stimulates 2,6-biphosphate fructose formation which influences the hormonal regulation of glycolysis and gluconeogenesis in liver [49]. The most important pharmacological significance of vanadium lies in glucose metabolism [50], lipoprotein lipase (LPL) activity [51], vanadate-dependent NADH oxidations-reductions [52], growth of red blood cells, adenylate cyclase activity and amino acid transport [53]. Vanadium at a low concentration takes a significant role in the modification of DNA synthesis and repair, but it appears cytotoxic at higher doses [21, 54]. The finding of unique properties of this inorganic micronutrient and its complexes may contribute in the maintenance of human health and development of therapy for the prevention of cancer.

8.4 *In Vivo* and *In Vitro* Studies

8.4.1 Breast

Fluid Ehrlich ascites tumor (EAT) appears as a spontaneous breast carcinoma in mice. Kopf-Maier and his group have established, among a group of metallocene dichloride, vanadocene dichloride (VDC) is capable of significant reduction of cell proliferation at very low concentration ($5–10 \times 10^{-6}$ mol/l) [55]. The antitumor activity of VDC against fluid EAT is reflected by accumulation in the nuclear heterochromatin [56], induction of mitotic aberration [57], transient suppression of mitosis, reversible cell accumulation in the late S and G_2 phase [58]. Vanadium

(IV) complex of 2-methylaminopyridine have been found to have similar activities on EAC cells like superoxide dismutase (SOD). M. M. El-Nagger et al. studied the antineoplastic effects of these complexes by intraperitonally administering them to Swiss albino mice infected with EAC cells. The value of EAC cells and EAC cell viability were found to be effectively decreased. Not only that, but activities of glutathione peroxide (GSH-Px) and glutathione reductase (GSH-R) also lowered along with significant elevation in the activities of SOD and glucose-6-phosphate dehydrogenase (G6PD) in Vanadium (IV) complex-treated mice [59]. A series of bisperoxovanadium compounds has been tested against DA3 murine mammary tumor model, in vivo. These compounds have also shown their efficacy against a panel of cell lines like MCF-7, NIH ADR, MB-231, MB-468 in vitro [60]. Metavan [bis(4,7-dimethyl-1,10-phenanthroline)sulfatooxovanadium] has been employed in the treatment of severe combined immunodeficient mouse xenograft models of human malignant glioblastoma and breast cancer and has been found to delay tumor progression, exhibit significant antitumor activity and prolong survival time [61]. In another study, it has been observed that vanadocene dichloride (VDC) and vanadocene acetylacetonate (VDacac) are effective chemical therapeutics against proliferation of glioblastoma cell lines and human mammary cancer cell lines. In a dose-dependent study VDC has been found to inhibit cell proliferation of BT-20 (breast cancer cell line), U373 (glioblastoma cell lines). The mechanistic study revealed that human cancer cell division is blocked by this organometallics through disruption of bipolar spindle formation [62].

In our laboratory, we have established the fact that the dietary supplementation of ammonium monovanadate in drinking water (0.5 ppm) causes significant reduction in tumor incidence, total number, multiplicity, size of the tumor cells in DMBA induced rat mammary carcinogenesis model [63]. Liver is the major organ for DMBA activation and detoxification [64]. This earlier information helps us to understand the underlying mechanism of anticarcinogenicity of vanadium. It acts through elevation of glutathione (GSH), glutathione-S-transferase (GST), cytochrome P450 (CYP) as well as inhibition of superoxide dismutase (SOD) and hepatic lipid peroxidation, indicating alteration of hepatic antioxidants along with phase I and phase II drug metabolizing enzymes. Later, in another study we have showed that only the mammary preneoplastic cells are sensitive to vanadium treatment, rather than the normal proliferating cells [65]. The additional information provided by this study was in connection with DNA repair through reduction of DNA protein cross-links, cell proliferation and induction of apoptosis. In a similar study, reduced metallothionein expression was observed immunohistochemically. Vanadium treatment slows down the hyperplasia occurrence and thus due to increased latency period delays the tumor appearance [66]. In another similar type of experiments, the effect of vanadium on DNA chain break was studied. In compare to DMBA control group, the DMBA+vanadium treated one gives almost 61% protection against single strand breaks. Vanadium (6.25 μM) imparts 62.9% protection against chromosomal aberration to DMBA induced cells [67]. The apoptogenicity of vanadium (100, 175, 250 μM) against human cancer cell line MCF-7 was studied by Chatterjee et al. through TUNEL assay and they confirmed

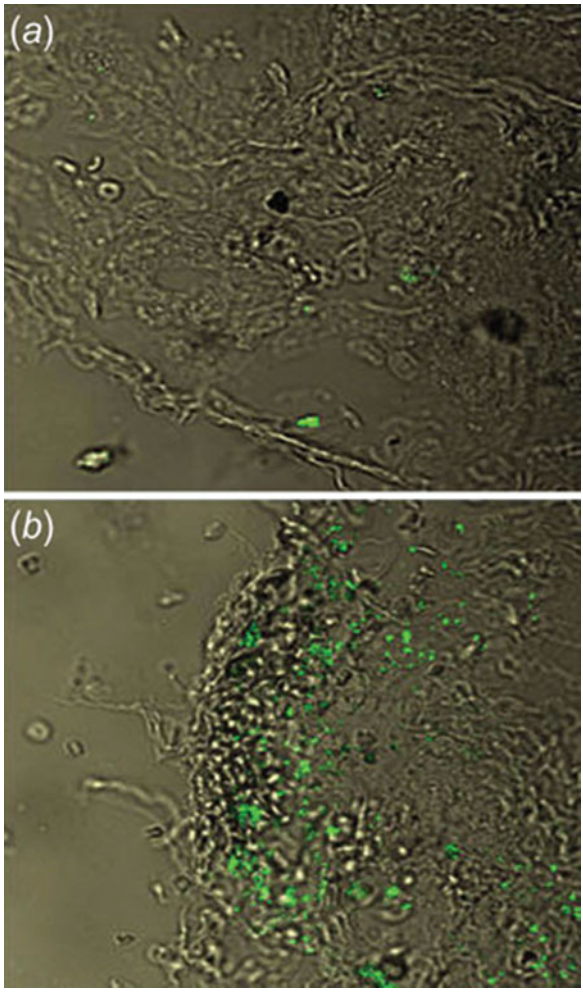

Fig. 8.1 Immunofluorescent localization of p53 in the mammary tissue of rats. p53 antibody was used at 1:100 dilution. (**a**) DMBA control (i.e., *group B*) stains very weakly for p53 and (**b**) DMBA with vanadium (i.e., *group C*) stains strongly for p53. Original magnification, ×100

that MCF-7 cells on vanadium treatment was likely to be apoptotic, rather than necrotic due to the presence of prominent apoptotic bodies [68]. Downregulation of Bcl2 and upregulation of p53 (Fig. 8.1), Bax are responsible for inhibition of cell proliferation and induction of apoptosis in vanadium treated DMBA induced rat mammary carcinogenesis (Fig. 8.2) [69].

8.4.2 Liver

The idea of considering vanadium as an effective chemopreventive agent against chemically induced hepatocellular carcinogenesis came from several studies. In our

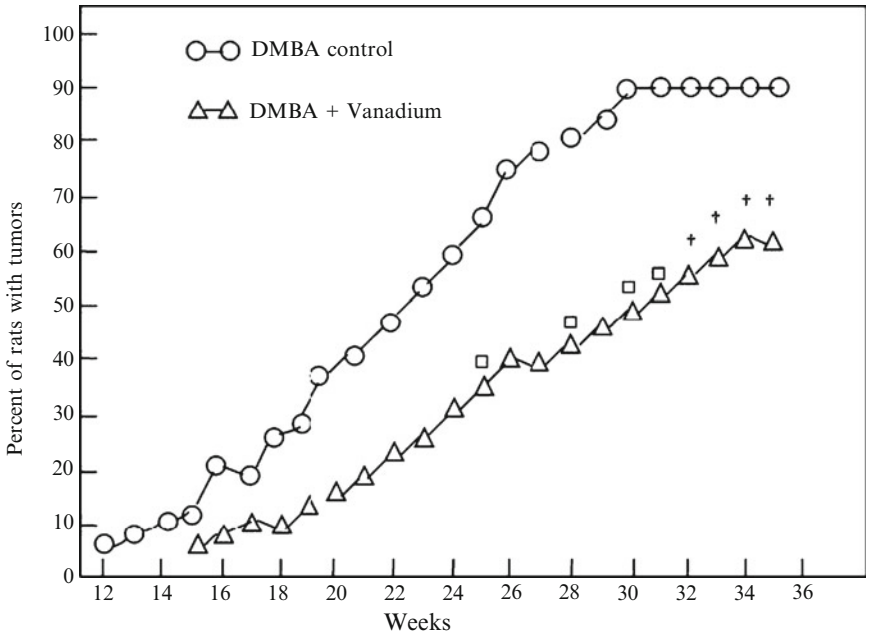

Fig. 8.2 Cumulative incidence of palpable mammary tumors (adenocarcinomas) in DMBA control (*group B*) as well as vanadium supplemented DMBA group (*group C*). DMBA was given to rats at 50 days of age at a dose of 0.5 mg per 100 g body weight via the tail vein. Ten animals were sacrificed for each time point following DMBA injection. Supplementation of vanadium in drinking water was started immediately after the carcinogen treatment and it continued for 35 weeks. □$p < 0.001$ and †$p < 0.05$ compared with the DMBA control (*group B*) by Fischer's exact probability test

laboratory we have studied the enhancement of activity of glutathione-s-transferase on oral administration of vanadium in a dose depended manner (100, 200, 400 nM for 30 days) in rat liver, kidney, small and large intestine mucosa. Moreover, this enhancement of enzyme activity is not associated with hepatic or renal toxicity as evidenced by glutamic pyruvic transaminase, serum glutamic oxaloacetate transaminase [70]. Some peroxovanadium compounds [bpv(Me_2Phen), bpv(OHpic)] have shown to be effective PTP inhibitors by Barry et al. [71]. In diethylnitrosamine induced hepatocarcinogenesis model of male Sprague-Dawley rats, supplementary vanadium (ammonium monovanadate) was given (0.5 ppm) to study its anticarcinogenicity. The vanadium treated group was found to have reduced incidence, number, multiplicity of hepatocytic nodules with respect to carcinogen induced group. Vanadium treated group also found to contain decreased number of altered liver cell foci (Table 8.1) [72]. In a phenobarbital promoted DENA induced hepatocarcinogenesis model of rats, supplementary vanadium has been proved to be potential therapeutic. The treatment group was appeared to have a decreased number of surface area of GGT-positive hepatocellular foci along with altered size distribution of visible persistent nodules [18]. In a similar study, it has been observed that DENA has

Table 8.1 Effect of vanadium supplementation (0.5 ppm) on the development persistent nodules in livers of rat initiated with DENA and promoted by PB

Group	No. of rats with nodules per total no. of rats	Nodule incidence (%)	Total no. of nodules	Average no. of nodules per nodule – bearing liver (nodule multiplicity)
A (carcinogen control)	10/10	100	383	38.3 ± 5.8[a]
C (carcinogen + vanadium for 20 weeks)	5/12	41.6[b]	52	10.4 ± 2.7[c]
E (vanadium treatment for 4 weeks before initiation)	7/12	58.3	136	19.4 ± 3.8[d]
G (vanadium supplementation 1 week after initiation and continued upto 20 weeks)	8/11	72.7	241	30.1 ± 4.7

[a]Each value represents the mean ± s.e.
[b]$P < 0.01$ as compared with group A by Fischer's exact probability test
[c]$P < 0.001$ as compared with group A
[d]$P < 0.02$

affected hematocrit value, RBC count, total WBC count, haemoglobin content with respect to normal. This adverse effect was found to be reversed in vanadium supplemented group. It also lowered plasma globulin content, prevented depletion of the plasma albumin concentration and thereby increased albumin to globulin ratio [73]. In order to find out a plausible mechanism of anticarcinogenic property of vanadium, it has been found that though it is not capable of altering the activities of hepatic phase I enzymes but of phase II ones like hydrocarbon hydroxylase, cytochrome P45062E1 (CYP2E1), UDP-glucoronyl transferase (UDPGT) [74, 75]. Chatterjee et al. further investigated the chemopreventive effects of vanadium in combination with vitamin D_3 against DENA – induced rat hepatocarcinogenesis model. Vitamin D_3 and vanadium in combination have been found to impart maximum protection against DENA-induced chromosomal aberrations and DNA strand breaks [76]. In another study their combinatorial effects reduced the number and area of placental glutathione-s-transferase positive altered hepatocyte foci (AHF) and nodules [77]. In the elevation of hepatic microsomal cytochrome P-450(Cyt. P-450), the combinatorial effect of vitamin D_3 and vanadium was found to be more efficient in contrast to their single effect. Furthermore, the combination also found to have significant role in reduction of GGT positive foci, cytosolic glutathione and glutathione-s-transferase (GST) [78]. A group of scientist from Poland synthesized V^{3+} complexes of cysteine, alanine and aspartic acid and exploited against hepatoma Morris 5123 cell lines. The probable route to cancer

cell death was assumed to be apoptotic [79, 80]. In a study conducted by our laboratory indicated sister chromatid co-efficient has significantly been reduced on vanadium supplementation through drinking water in a 2-acetylaminofluorene (2-AAF) induced rat hepatocarcinogenesis model [81]. The combined effect of vanadium and β-carotene has also been studied on DENA-PB induced rat liver carcinogenesis. In combination they imparted an additive effect in reduction of the expression of GST-positive AHF, the no. and size of hyperplastic nodules [82]. Vanadium has shown its anticlastogenic potentiality through inhibition of early hepatic cells. In addition, the GGT and PCNA expression were significantly reduced [83]. Later studies confirmed that the rise in the level of DNA base 8-OHdG, metallothionein, and trace elements in DENA-PB induced carcinogenesis and their significant reduction in vanadium supplemented group [84]. In another study from our laboratory, the elevation in the level of biomarkers like GGT positive foci, glycogen foci, PCNA, genotoxic DNA damage were investigated in chemically induced rat hepatocarcinogenesis and were found to be significantly lowered on vanadium treatment [85]. The anticlastogenic potential of this dietary micronutrient was established in a study where the formation of CA, SSB, DPC and tissue specific 8-OHdG are found to be inhibited by this dietary supplement [86]. The chemopreventive role of vanadium at the initiation stage of chemically induced hepatocarcinogenesis was investigated by the same author from our laboratory. The study confirmed that vanadium is capable of in limiting early molecular events and preneoplastic lesions through inhibition of DNA adduct and prevention of oxidative DNA damage in early stage of neoplasia [87]. In the expression of premalignant phenotype of the cell, metallothionein overexpression, DNA-protein crosses links, cell proliferation are found to have implicative role in rat liver preneoplasia. Vanadium as a chemopreventive agent has been found to have noteworthy impact on reduction of DPCs, CAs and MT immunoreactivity [88]. Dietary supplementation of vanadium is studied to have significant role on inhibition of formation 8-OHdG, DNA chain break along with in limiting cell proliferation in the initiation stage of neoplastic development in a two-stage (DENA-PB induced) hepatocarcinogenesis models [89]. In an in vitro study Na_2VO_3 has been established most effective against progressive growth of rat hepatoma H35-19 cells [90].

8.4.3 Colon

Colon cancer is one of most common type of epithelial malignancies [91]. Chemically induced rat colon carcinogenesis model has been studied several times in order to understand the chemopreventive effect of vanadium in this regard. In an earlier study vanadate, the potent mitogen was fed to 1,2-dimethylhydrazine induced mice colon carcinogenesis model. Vanadate at a concentration manner (10 or 20 ppm) was observed to elevate thymidine incorporation. But, it remains surprising non-influential in the development of large bowel tumors in DMH-treated

mice [92]. In our laboratory, we have carried out an investigation to establish the chemopreventive role of vanadium on DMH-treated colon carcinogenesis model in rats. We have observed that vanadium supplemented group (ammonium monovanadate, 0.5 ppm) contained reduced number of aberrant crypt foci (ACF), few colonic carcinomas and adenomas in contrast to only DMH-treated individuals. In addition, vanadium on one hand, elevated cyt p-450, liver GST activities, on other hand lowered PCNA index in ACF [93]. The study was further extended where we have seen the inhibitory effect of vanadium on the overexpression of GST-P positive foci. Additionally SOD activities in both liver and colon were found to be elevated upon vanadium treatment [94]. In another study it has been shown that the vanadium supplementation in drinking water significantly reduced the extent of DNA damage and chromosomal aberrations in colon cells, along with the activities of glutathione reductase and catalase [95]. The inhibitory effect of vanadium on DNA-protein cross-link and surface levels changes of ACF was well established in an in vivo study from our laboratory [96].

The individual PTP inhibitors orthovanadate and bpV(Phen) completely abrogated both the adenosine suppression of ERK1/2 activation and downregulation of cells surface DPPIV on HT-29 colon cancer cells [97]. In an in vitro study, synthesized VOHesp complex has exhibited its anticarcinogenicity against human colon carcinoma cell line Caco-2. The antioxidant activity of hesperidin complex has been enhanced in VOHesp as it became a better SOD mimic [98]. The fact that vanadium is an effective chemopreventive agent against DMH-induced rat colon carcinogenesis model is well established. Further studies have been performed to investigate the mechanism of its action of chemoprevention. O^6 – methylguanine (O^6-Meg) is a potent mutagen in DMH-induced colon cancer. Upon treatment with vanadium, the DNA adducts, O^6-Meg formation has significantly been lowered in contrast to the DMH-treated rats. Nuclear immunoexpression of p53 in vanadium treated group in compare to the extranuclear, random, irregular overexpression of p53 in DMH-control group supports for inhibitory effects of vanadium. Moreover an effective positive correlation between AI & p53 expression was observed in vanadium treated group rather than DMH-control one and iNOS mRNA expression has also significantly been reduced (Fig. 8.3) in vanadium supplemented group [99]. Vitamin D_3 is a well studied carcinogen inhibitor. Investigation was carried out in our laboratory to study the combinatorial effect of vitamin D_3 and vanadium in carcinogen induced rat colon cancer model. Fewer BrdU positive cells in vitamin D_3 and vanadium treated group (Group B, C and D) shows (Fig. 8.4) reduced BrdU immunopositivity when their carcinogen assaulted counterpart come in comparison. Vanadium and vitamin D_3 in combination has been established as effective inhibitor of colonic O^6 -Meg formation (HPLC fluorescence assay). Their combinatorial effect against DNA strand break also needs to be mentioned. The duo on one hand inhibits cell proliferation by downregulating antiapoptotic protein Bcl2 (Fig. 8.5) on the other hand it triggers apoptosis via elevation of p53 immunoexpression [100].

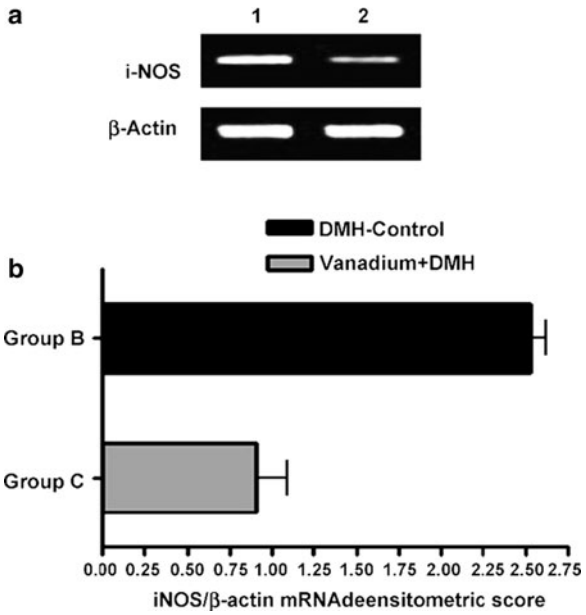

Fig. 8.3 RT-PCR analysis of iNOS mRNA expression in colon tumors. (**a**) RTPCR analysis of 30 cycles showing the representative blots of iNOS mRNA transcripts (*upper bands*) and _-actin mRNA transcripts (*lower bands*) in colonic tumor tissues from DMH control (*lane 1*) and vanadium treated (*lane 2*) animals. (**b**) Densitometric quantitaions of the agarose gel band is presented as a bar diagram, and the band intensities represented in the form of ratio of densitomentric scores (iNOS/_-actin). Mean ratio and S.E. were calculated from the signal obtained from ten different samples in each group. iNOS mRNA levels (iNOS mRNA/_-actin mRNA) were significantly lowered in vanadium treated group (*group C*) than that of DMH controls (*group B*) ($P < 0.001$)

8.4.4 Prostate

Metavan, a most potent anticancer, multitargated vanadium complex, has been passed successfully against tumor cells derived from prostate cancer patients [61] Sodium orthovanadate (Na_3VO_3) and vanadylsulphate ($VOSO_4$) have been exploited successfully against human prostate (DU145) cancer cell line in a dose dependent manner. It has been suggested that vanadium as a pro-apoptotic factor affects the protein kinase activities through inhibition of phosphatases [101, 102].

8.4.5 Lung

Inhibitory effect of sodium orthovanadate against human epithelial A549 cancer cell line has been established by Klein et al. in an in vitro study [102]. Vanadium (III) –L-cysteine compound like [VIII$(Hcys)_3$]. 2HCL.2.5H_2O has been studied by a group of scientist in order to evaluate its antimetastatic effect against 3,4-benzopyrene treated Wistar rats [103].

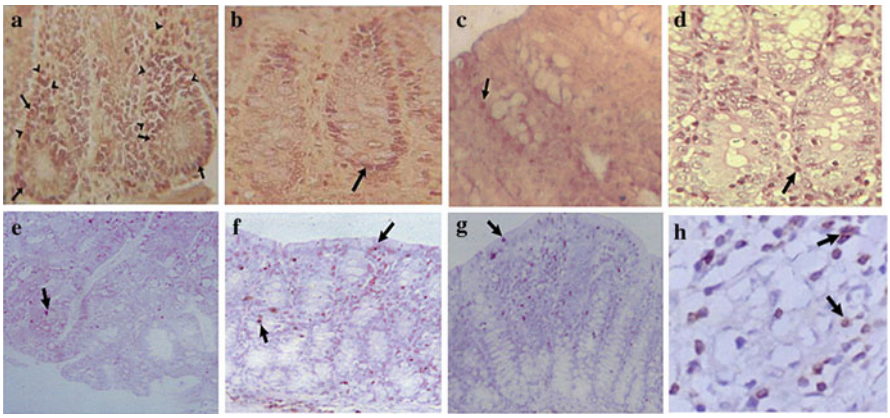

Fig. 8.4 Representative immunohistochemistry pictures of BrdU labeled cell proliferation and TUNEL positive apoptotic cells in colon tissues of rats in presence or absence of Vanadium (V) and/or 1, 25-dihydroxy-vitamin D_3 (Vitamin D_3). Brown-stained (*arrow*) cells are undergoing cell proliferation (**a**, **b**, **c**, **d**) or apoptosis (**e**, **f**, **g**, **h**). Approximately 50 crypt columns per site per animal were randomly chosen and 10 rats were examined per group. (**a**) (100×) represents DMH control group. Highly proliferative zones are marked by arrowhead; (**b**, **c**, **d**) (100×) respectively represents V-treated (*Group B*), vitamin D_3 treated (*Group C*) and V+Vitamin D_3 treated (*Group D*) DMH group. (**e**) (40×) represents DMH control group with few TUNEL positive cells in colonic crypts. (**f**, **g**) (40×) and (**h**) (150×) respectively show colonic crypts with TUNEL positive cells from V, Vitamin D_3 and V+Vitamin D_3 treated group

8.4.6 Pancreas

Vanadate with somatostatin causes dephosphorylation of membrane proteins through inhibition of epidermal growth factor(EGF). This may be the probable mechanism of inhibitory effect of human pancreatic cancer cell as evidenced in MIA PaCa-2 cell line [104].

8.4.7 Bone

Most of the vanadium we intake is accumulated in our bone which generate a concept of using vanadium in the treatment of bone tumor metastasis. Vanadium(IV) being complexed with aspirin using poly(β-propiolactone) as delivery system has been successfully used against UMR106 osteosarcoma cells to investigate its antineoplasticity [105]. Cytotoxity of other vanadyl and pervanadate has been evaluated against osteoblast (MC3T3E1) and osteosarcoma (UMR106) cell line in vitro [106]. Vanadium in a dose dependent manner induces ROS formation, cytotoxicity and thiobarbituric acid reactive substances (TBARS) formation in the

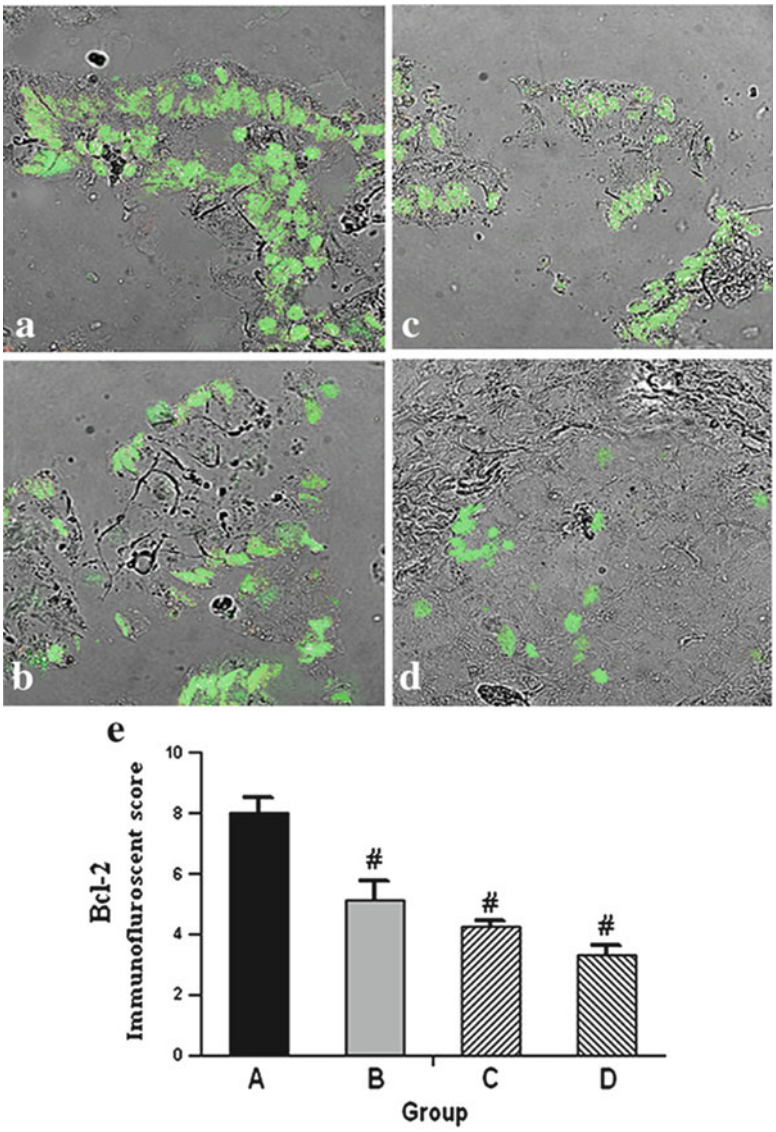

Fig. 8.5 Bcl-2 immunofluorescence in colon tissues of rats. (**a**, **b**, **c**, **d**) Immunofluorescent localization of Bcl-2. Bcl-2 antibody was used at 1:400 dilutions. (**a**) DMH control (*Group A*) stains very strongly for Bcl-2 and (**b**, **c**, **d**) DMH with vanadium (V) and/or 1, 25- dihydroxy-vitamin D3 (Vitamin D3) (*Group B*, *C*, *D*) stains weakly for Bcl-2 with a gradual decrease of expression in V+DMH (**b**), Vitamin D3 + DMH (**c**) and V+Vitamin D3 + DMH (**d**) treated group respectively. Original magnification, ×200. (**e**) Graph represents Bcl-2 immunofluorescent score in DMH challenged colon tissues in presence or absence of vanadium and/or vitamin D3. Each bar represents the mean value ± S.E calculated from 8 slides/rat and 10 rats/group. #Pb0.001 when compared to DMH control group

concerned cells. In a recent study, the fact has been established that the key events of tumorigenesis, cell adhesion, migration and clonogenicity have been inhibited by TreVo, GluVo in UMR106 osteosarcoma cells [107].

8.5 Epidemiological Study

Fallico et al. carried out a longitudinal retrospective case-control study on the populations of the Etna massif as a consequence of vanadium assimilation through diet and its effects on diabetes mellitus, heart arrhythmia, renal lithiasis and arterial hypertension. They hypothesized that vanadium has a protective role on the mentioned pathologies. It was observed in this study that the healthy controls have turned out to have higher concentrations of V in the blood and urine compared to the cases. Further and more longitudinal studies are needed on representative groups to verify and confirm their hypothesis [108]. A team led by Jaroslav Lener have performed a 3-year follow-up of some indicators like hematological and cytogenetic status, cellular and specific immunity, sperm lipids level in some school children (10–12 years) exposed to vanadium pentoxide emission from metallurgical plant in their vicinity. The finding is long-term exposure to vanadium emissions had no negative impact on their health. Furthermore, significant higher values of T-lymphocyte mitotic activity in children from immediate proximity of the plant producing vanadium indicated that vanadium have an elevated activity of cellular immunity [109].

8.6 Mechanisms of Action

Micronutrients like vitamin D, lycopene, folate, calcium are currently undergoing clinical trials to investigate their pharmacological potentiality [110]. Selenium is the most evidenced inorganic nutrient which has happened to be a promising chemopreventive agent. Although, less known than selenium, vanadium is gaining importance for its potential chemopreventive effects [111]. Cancer morbidity and mortality were reduced when vanadium in its various forms placed in the diets of the experimental model [17, 72, 73]. Daily supplementation of $VOSO_4$ (25 ppm) in diet inhibited induction of mammary carcinogenesis in experimental model and reduced both the number of cancer incidences and average number of tumors [72]. Further studies have shown its inhibitory effect on DMBA, DENA and DMH induced carcinogenesis. But, very scanty mechanistic data of its chemopreventive properties against cancer over a large population area is available. The well-known biochemical effect of vanadium elevation is decreased lipid peroxidation leading to an increase in oxidative protection processes [63]. The other mentionable biochemical activities are elevated cytochrome P450 expression, increased glutathione level and glutathione-S-transferase activity, lowered superoxide dismutase activity and last but not the least, elevation of Phase II conjugated enzymes; UDP-glucose

dehydrogenase, UDP-glucoronyl transferase [63, 74, 112]. Possibly, it also elevated the detoxifying enzyme activities. Due to presence of structural similarity to PO_4^{3-}, VO_4^{3-} interacted with phosphate processing enzymes and participated in metabolic pathways of carbohydrates and lipids. All these together highlighted the beneficial activity of vanadium.

Vanadium supplementation in diets of chemically induced carcinogenesis model is a result of minimized DNA instability – evidenced from a number of studies. But, no suggested mechanisms account for the decreased DNA alkylation. Vanadium predominates as oxoanions (VO_4^{3-}) in aqueous solution [113–115] and thus exhibit nucleophilic character which in turn attack the electrophilic alkylating agent. The ability of vanadium salts in the inhibition of DNA damage, when subjected to an alkylating assault was demonstrated by Wilker et al. The fact that oxospecies such as $V_3O_9^{3-}$ detoxified alkylating agent through hydrolysis to yield corresponding alcohols was revealed from NMR-spectroscopic analysis. For example, vanadates transformed ethyl iodide (CH_3CH_2I) and $(CH_3CH_2O)SO_2$ to ethanol (CH_3CH_2OH) [115].

It is evidenced that Na_3VO_4 gives greater protection to DNA alkylation than Na_3SeO_4. This is because VO_4^{3-} is a better nucleophile than SeO_4^{3-} due to presence of greater charge density. Vanadate brings equilibrium between various species, depending upon concentration, solution pH and oxygenation levels. We cannot say conclusively that this "carcinogen interception" mechanism exactly occur within cells. Only a significant interaction between inorganic species and alkylating carcinogens is discussed here.

Some other possible mechanisms proposed by the scientists are increased activity of phosphodiesterase [116], inhibition of protein phosphatases [117], or generation of reactive oxygen species and oxidative stress [118].

8.7 Toxicity vs. Beneficiary Action

Toxicity of vanadium compounds to the exposed groups depends on its dose, route of administration, time span of exposure and nature of the compound itself [2, 111]. A series of toxicity and pharmacokinetic studies have been performed on experimental animal models, but very scanty data are available on clinical studies and long-term effect on humans. In a clinical trial, the effect of $VOSO_4$ was studied in 31 weight training athletes. There was no significant effect of treatment on blood viscosity, hematological indices and biochemistry [119]. Oral administration of $NaVO_3$ has shown minimal side-effects like mild gastrointestinal intolerance [120]. Twelve healthy adults were orally administered vanadium (125 mg/day) in the form of diammonium vanadotartarate to investigate its effect on serum cholesterol effect. But, no changes in levels of serum cholesterol, lipoprotein pattern, haemoglobin, blood urea were observed [121]. Another study was performed to investigate the toxicity of ammonium vanadyltartarate administered orally to human subjects. Only cramps and diarrhea were observed where high-doses (4,325, 4,225 mg) were

given to them. No statistical changes occurred in triglycerides, cholesterol, blood lipids, phospholipids which is indicative of the absence of toxicity. Unpredictable vanadium absorption is suggested by varying amounts of vanadium excretion [122]. Oxytartarovanadate was administered at a level of 4.5 mg V/day for 16 months to patient, who showed no sign of toxicity throughout the study [123]. Vanadium is appeared to be more toxic when inhaled rather ingested. Vanadium pentoxide fume from several industries was proved to be causative agents of acute chemical pneumonitis, pulmonary oedema to acute tracheobronchitis [124]. In an epidemiological study, it has been established that inhalative exposure to vanadium pentoxide was responsible for DNA instability in workers from a vanadium pentoxide factory [125]. As an additional note, toxicity of vanadate and vanadyl salts was observed only at remarkably higher doses in compare to the usual amount ingested through diets. So it is evident, that under normal environmental and nutritional condition of exposure vanadium would not be hazardous to human health [5]. The effect of this transition metal on animal physiology and their individual response to the treatment might be influenced by plasma and cellular binding proteins (including catecholamines and glutathione), severity of diseases (including gastrointestinal and renal function), exercise, stress and genetic predisposition [126]. These factors would be accounted in determining proper dose, route and time of exposure of vanadium compounds in cancer treatment. Therapeutic advantage of oral administration, and available options for lessening toxicities warrant development of safe and effective pharmacological formulations.

8.8 Conclusions

An objective of this review has been to provide comprehensive picture of current biological, chemical and clinical research on vanadium. There is heightened need to draw together the numerous threads and present a coherent picture of the various research endeavors.

The present study demonstrates vanadium mediated inhibition of different forms of cancer and illustrates an emerging concept of chemoprevention through inhibition. Vanadium functions as an inhibitor of cancer through modulation of cell proliferation and apoptosis. Authors suggest that by induction of oxidative stress or inhibition of phosphatases. Ortho or metavanadates affects the activities of protein kinase which are adjusted during cell growth. An additional question is whether the antiproliferative effect of vanadium will allow employing such compounds as auxiliary drugs in certain types of cancer. The extensive use of this element together with its complex chemistry and the narrow threshold between essential and toxic doses makes it crucial to understand as much as possible about vanadium. Ultimate determination of essentiality for human will depend on the greater understanding of the fundamental biochemical roles of vanadium.

There is however no studies or reports on the pharmacokinetics of vanadium metabolism in human subject and thus additional studies are warranted to determine

the optimum effective dose of vanadium in inhibiting cancer in human. The need of the moment is to improve the experimental design, interpret the observed effect and encourage their use as templates for further development of more effective and safe chemopreventive compound.

The data summed up here add another layer to the already highly complex word of vanadium that could prove to have a stronger antitumor activity.

However we need to collect significant information from experimental, clinical, and epidemiological result before we support any public health recommendation. To do this we need more research and more exchanges within the scientific communities of basic and applied research disciplines.

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