Vanadium, a Versatile Biochemical Effector in Chemical Rat Mammary Carcinogenesis

Rajarshi Sankar Ray, Mitali Basu, Balaram Ghosh, Kartick Samanta, and Malay Chatterjee

Abstract: Recent studies indicate the role of the micro-nutrient vanadium in chemoprevention in many animal models, human cancer cell lines, and also in xenografted human carcinomas of the lung, breast, and gastrointestinal tract. The present studies were undertaken to ascertain the antineoplastic potential of vanadium in a defined model of mammary carcinogenesis. Female Sprague-Dawley rats, at 50 days of age, were treated with 7,12-dimethylbenz(α)anthracene (DMBA) (0.5 mg/100 g body weight) by a tail vein injection in oil emulsion. Vanadium (ammonium metavanadate) at a concentration of 0.5 ppm (4.27 µmol/l) was supplemented in drinking water and given ad libitum to the experimental group after the carcinogen treatment, and it continued until the termination of the study. In vivo studies of DNA chain breaks demonstrated that vanadium offered significant (61%, P < 0.005) protection against generation of single-strand breaks when compared with the DMBA control group. Supplementation of vanadium normalizes the level of zinc, iron, and copper as revealed by proton-induced X-ray emission analysis to a substantial extent (P < 0.01). In vitro study of chromosomal aberrations (CAs) revealed that vanadium triggered a protective effect (62.9%) on induction of CAs, which was maximum on structural aberrations followed by numerical and physiological types. Histopathological and morphological analyses were done as end-point biomarkers. We conclude herein that vanadium has the potential to reduce genomic instability in mammary carcinoma in rats.

Introduction

Vanadium is considered to be a micronutrient and is included in the list of 40 essential micronutrients that are required in small amounts for normal metabolism. Accordingly, it has been incorporated in many multinational pharmaceutical preparations (Nutrition Dynamics, Inc., Seguin, TX; All Nature Pharmaceuticals, Inc., City of Industry, CA; and Ranbaxy Pvt. Ltd., Mumbai, India.) along with vitamins and other essential trace elements for maintenance of normal health. Although micronutrients do not have pharmacological potencies, they prevent regular wear and tear of the essential critical molecules of the cell such as DNA, proteins, etc. (1). Vanadium has a role in the DNA maintenance reaction and may protect the genomic instability that may lead to cancer. Further, vanadium has a definite positive role in carbohydrate metabolism, especially the insulin-mimicking activities (2,3). Vanadium is present in oils, fats, fruits, vegetables, cereals, liver, fish, spinach, oysters, shellfish, black pepper, and parsley.

Vanadium has shown documentary roles in chemoprevention in mouse tumor models (4), hepatic cancer (5), rat mammary cancer models (6,7), leiomyosarcomas in Wister rats (8), many human cancer cell lines (9), and also in xenografted human carcinomas of lung, breast, and gastrointestinal tract (10). Several other reports on vanadium and vanado compounds have indicated that low doses of vanadium are beneficial (11,12) compared with toxic higher doses (13).

It has long been recognized that in vivo exposure to exogenous mutagens and carcinogens results in increased gene mutation and chromosomal aberrations (CAs) in humans (14). Only recently it has been recognized that micronutrients, vitamins, and minerals are acting as substrates or co-factors in many DNA maintenance reactions (15), and thus their exact concentrations in cells may be critical. Micronutrient deficiency can mimic radiation (or chemicals) in damaging DNA by causing single- and double-strand breaks or oxidative lesions or both (16). CAs such as double-strand breaks have been studied as a strong predictive factor for human cancer (17).

7,12-Dimethylbenz(α)anthracene (DMBA)-induced experimental chemical carcinogenesis is particularly useful for rat mammary tumor studies. This model is widely used for animal experimentation because rat mammary glands develop neoplasm that closely mimics human breast disease and share several morphological similarities; for example, carcinogenesis initiation occurs primarily, as does human breast cancer, from the terminal ductal-lobular unit. Again, experimental conditions and requirements are well established and accepted in this model system. Furthermore, most of the lesions found in the human breast have a counterpart in rat pathology (18).
In this DMBA mammary tumor model induced in the rat, we tried to correlate the CAs and DNA chain break with levels of essential trace elements studied by proton-induced X-ray emission (PIXE) analysis. Histopathological and morphometric studies were taken as end-point biomarkers.

Metals are necessary for the normal functioning of cells, and their levels in vivo may be crucial for the survival of organisms. Ionic changes occur inside normal cells during progression through the cell cycle as well as in cells that have undergone transformation. The intracellular ionic environment plays a critical role in cellular functioning. Both normal and malignant cell activity is more susceptible to ionic concentrations inside them (19). Biologists have tried to obtain more information about the distribution of elements at the cell and tissue level using different methods. Since its introduction PIXE has been successfully used by various groups through-out the world for element analysis due to its high sensitivity, multi-elemental capability, and nondestructive nature.

Studies have shown that metals that undergo redox cycling and deplete glutathione and protein-bound sulfhydryl groups produce reactive oxygen species (ROS) such as superoxide ion, hydrogen peroxide (H2O2), and hydroxyl radical. As a result, enhanced lipid peroxidation, DNA damage, and altered calcium and sulfhydryl homeostasis occur (20). Evidence suggests that much endogenous DNA damage arises from intermediates of oxygen reduction, which then attack the bases or the deoxyribosyl backbone of DNA or can attack several components such as lipids to generate reactive intermediates that couple to DNA bases. Endogenous DNA lesions are genotoxic and induce mutations, CAs, and DNA damage.

Information on the effect of vanadium and its antineoplastic potential is still meager. In this article, we report for the first time that vanadium can modulate the activity or concentration of other elements and thereby tend to stabilize the genome from DNA damage induced by DMBA in rat mammary carcinogenesis.

### Materials and Methods

#### Chemicals

Dulbecco’s modified Eagles’ medium (DMBA), fetal bovine serum (FBS), DNase I, type II collagenase, and Ficoll were obtained from Sigma Chemical Co. (St. Louis, MO). Ammonium metavanadate (NH4VO3, +5 oxidation state) was purchased from E. Merck (Mumbai, India). DMEM was purchased from Hyclone, Inc. all other chemicals and reagents used were of analytical grade and purchased in the purest form from local firms.

#### Animals and Treatment

Inbred virgin female Sprague-Dawley rats obtained from the Indian Institute of Chemical Biology (Kolkata, India) were used for the experiments. Animals of 40 ± 2 days were housed in plastic animal cages (Tarsons, five rats per cage) in a room regulated for temperature (25 ± 1°C) and humidity (50–60%) and exposed to a 12:12 h light/dark cycle. All rats were fed a semipurified basal diet and demineralized water ad libitum. The rats were acclimatized to the laboratory conditions for a period of 10 days before the start of the experiment.

### Experimental Design

Animals were divided as

- **Group A:** Normal control animals given a single tail vein injection of corn oil emulsion vehicle at 50 days of age.
- **Group B:** DMBA-treated animals that served as carcinogen control.
- **Group C:** Rats treated with DMBA and then provided vanadium supplemented in drinking water (at 0.5 ppm, equivalent to 4.27 µmol/l) ad libitum.
- **Group D:** Rats treated with corn oil emulsion and then provided 4.27 µmol/l vanadium in their drinking water ad libitum (vanadium control) (Fig. 1).

For morphological study, 260 animals were assigned to each of the 4 groups, and 10 animals from each group were sacrificed for each time point. However, for histological PIXE analysis and for DNA chain break studies, each group had five animals. When the animals were 50 days of age DMBA at a dose of 0.5 mg/100 g body weight in corn oil emulsion was given to the animals of Groups B and C as a single tail vein injection (21). Vanadium treatment was started immediately following the carcinogen or vehicle administration and continued until the termination of the experiments.

A stock solution of NH4VO3 was made by dissolving 50 mg of NH4VO3 in 100 ml of double-distilled deionized water. The final working solution was obtained by adding 1 ml of the stock solution to 1 l of double-distilled deionized water.

<table>
<thead>
<tr>
<th>Exp. Wks</th>
<th>0</th>
<th>4</th>
<th>24</th>
<th>35</th>
</tr>
</thead>
</table>

**Figure 1.** The basic in vivo experimental regimen.
The concentration of the working solution was 4.27 \mu mol/l. The pH was adjusted to 7.2. It was given as drinking water to the Group C and Group D animals ad libitum and changed daily. The 4.27-\mu mol/l dose of NH4VO3 in the drinking water was found to be nontoxic (7,22,23). Treatment was continued until 24 wk for histological PIXE analysis and DNA chain break studies and up to 35 wk for morphological findings. The rationale of such experimental end points has been described in detail elsewhere (21). The body weights of the animals were measured twice per week throughout the experiment; water and food intakes were recorded daily. The animals were palpated twice a week to observe the development of palpable mammary tumors, and the time of tumor appearance was also recorded.

**Morphological Study**

Ten animals from each group were sacrificed each week starting at 10 wk after DMBA injection and continuing to 35 wk. The animals were anaeasthetized with ether and opened by midline incision from the pubis to the submaxillary area. The skin was dissected out to expose six pairs of mammary glands. Any gross modification of the mammary fat pad by vascularization or presence of any palpable as well as nonpalpable tumors was recorded. Only well-developed adenocarcinomas, confirmed through histology according to the criteria of Young and Hallowes (24), were reported. Tumors were removed surgically, their sizes were measured with the help of a Vernier scale, and the weights were also recorded. The tumors were divided and analyzed according to their sizes as reported previously (21).

**Histological Evaluation of Mammary Tissue**

Twenty-four weeks after the carcinogen or vehicle treatment, animals from each group were randomly selected, and the thoracic and abdominal inguinal mammary tissues were surgically excised from ether-anaesthetized rats. The recovered tissues were then fixed in 10% formalin and processed for histological studies. The tissues were dehydrated through 70%, 90%, and 100% alcohol and embedded in low-melting-point paraffin. Sections 5 \mu m thick were cut and placed serially on glass slides. The sections were deparaffinized in xylene and rehydrated through 100%, 90%, and 70% alcohol, and three contiguous sections were made from each mammary tissue and stained with hematoxylin and eosin for histological evaluation under light microscope. The histological slides were coded so that the particular sample identity was unknown to the individual performing the assessments.

**Experimental Setup for PIXE Analysis**

Approximately 5 ml of whole blood samples from three different groups (normal control group, DMBA control group, and vanadium-treated DMBA group) were collected and lyophilized at the Regional Medical Research Centre (Bhubaneswar, India). The samples were converted into fine powder, homogenized, and finally pressed into thin layer pellets. Conversion of the sample into fine powder before pelletizing is necessary to avoid particle size effects in the analysis. The thin-layer pellets were uniformly deposited over Mylar films of 3–6\mu m thickness. Yttrium was added as internal standard. Thick-target PIXE analysis was performed using GUPIX-95 software, which provides nonlinear least-squares fitting of the spectrum, together with subsequent conversion of the X-ray peak intensities into elemental concentrations via a defined standardization technique involving fundamental parameters and user-defined instrument constant. Full account was taken of matrix effects and secondary fluorescence contributions in both the spectrum fitting portion and the calculation of concentrations. Mean concentrations of essential/trace elements in \mu g/ml were measured by the PIXE analysis of blood samples.

**In Vitro Culture**

Virgin female Sprague-Dawley rats (45 days old) were killed by ether anesthesia. The abdominal and inguinal mammary glands were aseptically removed, pooled, transferred to DMEM containing 1% FBS and 0.45% type II collagenase, and incubated at 37°C for 60 min. The mixture was filtered through 157-mesh nylon to remove all dissociated tissues. The filtrate was incubated in the presence of 10,000 U/10 ml DNase I at 37°C for 10 min, after which cells were pelleted by centrifugation at 400 g for 10 min. The cells were suspended in DMEM containing 10% FBS and layered into a continuous 2–8% Ficoll gradient that rested on a cushion of 20% Ficoll. The cells were centrifuged at 70 g for 5 min at 4°C. Mammary epithelial cells migrated to the bottom 10 ml of the 90-ml gradient. These cellular aggregates were counted using trypan blue exclusion and stored in liquid nitrogen according to the process of Greiner et al. (25).

Mammary epithelial cells were thawed and grown in DMEM containing 5% FBS, 0.5 \mu g insulin, 0.5 \mu g cortisol, 0.5 \mu g bovine prolactin, 30 ng progesterone, and 0.3 ng/ml 17 \beta-estradiol. For passaging the cells and in the assay for CA, approximately 3–5 \times 10^6 mammary epithelial cells were seeded per 100-mm plastic dishes.

**Cell Viability Assay**

Cell viability was measured using Intergen’s Procheck cell viability assay (Purchase, NY) based on the conversion of XTT (sodium 3,3′-{1-[(phenylamino) carbonyl]-3,4-tetrazolium] bis (4-methoxy-6-nitro)-benzene sulfonic acid) from an oxidized tetrazole to a reduced formazan (26). Cells were seeded into 96-well plates with 10^4 cells per well and allowed to attach for 24 h. Cells were treated with 1.56–25 \mu M of vanadium (NH4VO3) for 24 h and then washed twice with PBS to stop the treatment. To the wells, 100 \mu l of media and 20 \mu l of assay reagent were added, and cells were incubated under growth conditions for 4 h. Optical densities were read at 475 nm in a microplate
spectrophotometer, and cell viability was expressed as a percent of control.

Assay for Chromosomal Aberration

When cells reached a confluence of 50–60% in the plastic dishes, DMBA, initially dissolved in acetone, was diluted in serum-containing DMEM and added to a final concentration of 0.25 µg/ml. Immediately, 50% of the cells were treated with 6.25 µM NH₄VO₃ dissolved in DMEM. After 24 h, cells that received the vanadium treatment and those which did not were washed twice with PBS to stop the treatment or the carcinogen reaction. Both sets of cells were then passaged every 5 days thereafter; after the sixth passage, the cells in each group were subcultured 1:5 and used 24 h later for chromosomal preparation. Colcemid was added 4 h before harvesting. The cells were mechanically detached from the surface of the dishes and exposed to 0.075 M KCl for 15–20 min. The cells were fixed with a 3:1 mixture of methanol/acetic acid, and the fixative was changed three times before preparing slides. Slides were prepared by spreading fixed cells over chilled (50% methanol) grease-free glass slides and put through a flame. The slides were kept overnight under air and stained with Giemsa (3% solution, pH 5.9) for 30 min for scoring chromosomal anomalies. The frequency of CAs was expressed as the percentage of total aberrant metaphase plates. Aberrations were classified into three major groups according to Sarkar et al. (27).

Assay of DNA Unwinding

DNA was isolated from the frozen mammary gland of rats of all the groups (that is, A, B, C, and D) by a modification of the published procedure of Gupta (28) with enzymatic RNA digestion before proteinase-K treatment of the tissue homogenate. DNA concentration and its purity were estimated spectrophotometrically (29,30) and then the solution was stored at −20°C.

The DNA unwinding assay was performed according to the published procedure of Sarkar et al. (27). Isolated DNA solution from each group was divided equally among three sets of tubes. The contribution to fluorescence by components other than double-stranded DNA (including free dye: ethidium bromide) was estimated from a blank sample (B) in which the DNA solution was first sonicated highly and then treated with alkali under conditions that cause complete unwinding of low molecular weight double-stranded DNA. A second sample was used for estimating total fluorescence (T), that is, fluorescence due to the presence of double-stranded DNA plus contaminants. The difference (T – B) provided an estimate of the amount of double-stranded DNA remaining. The percentage \( D \) is given by the equation:

\[
\text{Percentage } D = \frac{(P - B)}{(T - B)} \times 100
\]

where \( S \) is the percentage of DNA that remains single stranded after alkali treatments and \( D \) is the percentage remaining as duplex DNA. \( D/S + D \) represents the fraction \( f_0 \) of molecules without strand breaks. The values of \( n \) corresponding to different DNA solutions isolated from different groups (i.e., A, B, C, and D) were then estimated.

For this estimation, DNA was first sheared by passing the DNA solution (20–25 times) through a 24-gauge needle using a hypodermic syringe and then treated with alkali and neutralized. The extent of DNA unwinding after a given time of exposure to alkali was calculated from the fluorescent values of \( T, P, \) and \( B \) samples. The percentage of \( D \) (double-stranded DNA) is given by the same equation mentioned earlier.

Results

General Observations

During the entire study no treatment-related alteration in the daily intake of food and drinking water of animals was observed. This may indicate that treatment with vanadium did not exert a toxic effect. The growth of animals was not affected during the entire study of any of the treatments as no significant difference in final body weights against normal was observed (data not shown). The body weights of carcinogen control animals (Group B) were significantly higher than normal control animals (Group A) (data not shown). This can be attributed to the tumor growth in carcinogen control animals.

Morphology and Morphometry

In the DMBA control group, 100% of animals had tumors, with an average of 7.8 tumors per tumor-bearing rat (tumor multiplicity) at the end of 35 wk (Table 1). The position of each palpable tumor was marked with Bouin’s solution when detected. Over the course of the postexposure periods, tumors that did arise did so individually and not as multiples at the same time. Mean latency period of tumor appearance was 11.7 ± 0.1 wk. The majority of tumors appeared between 15 and 16 wk, and multiple tumors of different sizes were frequently found in the same animal; most of the tumors were between 2 and 5 mm in size (Table 2). Vanadium supplementation significantly \((P < 0.05)\) reduced the tumor incidence (Table 1), with rats in Group C showing a 60% tumor
incidence after 35 wk. Mean latency period of tumor appearance in this group was 16.2 ± 0.1 wk, a value significantly longer (P < 0.001) than that in Group B rats (11.8 ± 0.2). Tumor multiplicity was reduced by 36%, and there was a decrease in the percentage of rats developing tumors as the experiment progressed among the DMBA+vanadium–treated group. For example, between 25 and 31 wk, there was a highly significant (P < 0.001) lowering of tumor incidence.

Cotreatment with vanadium also attenuated the number of tumors that grew to 2–5 mm in size, indicating a slower tumor progression. No tumors were observed in the normal (Group A) and vanadium (Group D) controls at any time point.

Tumor Histology

Histological evaluation of mammary tumors was conducted on the 30th and 35th wk after carcinogen administration in the DMBA-treated group (Group B). The histological evaluation of the mammary tumors at the 30th wk revealed that the tumors were either adenocarcinomas or fibroadenomas. The histological evaluation of the mammary tumors at the 35th wk in the same group revealed that the tumors were invasive adenocarcinomas and adenocanthomas with squamous differentiation. The tumor cells lining the cavities underwent squamous differentiation or metaplasia with keratinization (figure not shown).

Mammary Histology

As depicted in Fig. 2A, the normal control (Group A) showed preserved normal ductular and alveolar structure of mammary tissue with uniform-looking epithelial cells. Mammary tissue from the DMBA-treated group (Group B) showed hyperplasia of the lobules recognized by a typical arborization and formation of groups of cells that line the dilated ducts of the alveolus (Fig. 2B). The cellular architecture was found to be altered, and enlargement of the alveolus with cells showing nuclear pleomorphism characterized by nuclear enlargement, clumping of chromatids, and prominent nucleoli was observed. Atypical epithelial hyperplasia was observed. Epithelial cells showed variation in nuclear size with irregular chromatin and prominent nucleoli. However, the myoepithelial layer was preserved in the alveolar structure, and it could not be considered frank carcinoma at this stage (Fig. 2B). It was observed from histological examination that the vanadium-treated DMBA group (Group C) presented a histological profile similar to that of the normal control (Fig. 2C). In this group there was mild ductular proliferation with focal epithelial hyperplasia. Epithelial cells were uniform in size, and anisonucleosis and mitosis were absent. Periductular adipose tissue was present with patchy inflammatory cellular infiltrate (composed mainly of lymphocytes). The vanadium control (Group D) showed no observable distinct change from the normal control (figure not shown).

Effect of Vanadium on Essential Trace Elements

PIXE analyses were used to determine the mean blood concentrations of essential trace elements in samples from rats in each of the treatment groups (Table 3). The results
Figure 2. A: Mammary tissue showing normal architecture at 24 wk post-oil emulsion injection (hematoxylin and eosin, H&E). ×100. B: Mammary tissue showing carcinogen control group at 24 wk post–DMBA (0.5 mg/100 g body weight) single injection by tail vein (H&E). ×100. C: Section of the DMBA-treated (0.5 mg/100 g body weight) mammary tissue showing almost normal architecture after continuous treatment of vanadium at the dose of 0.5 ppm ad libitum for 24 wk (H&E). ×100.
show that, although levels of copper and zinc were lower in the DMBA-alone rats compared with those in the normal controls, levels of iron were elevated. In contrast, blood samples from rats that received DMBA+vanadium exhibited a marked similarity to those of the normal controls. Profiles for the vanadium-only rats did not show any marked differences from untreated rats.

**Effect of Vanadium on DNA Chain Break Assay**

From the in vivo studies it was revealed that there was a significant rise in total percentage of DNA single-strand breaks in Group B animals when compared with Group A (Fig. 3). The percentage of native double-stranded DNA decreased in Group B animals by 3-fold ($P < 0.001$), and the total aberrant single-strand regions increased more than 10-fold ($P < 0.001$) than normal control in Group A. This shows the DNA-damaging efficacy of DMBA. In contrast, a statistically significant ($P < 0.001$) decrease in total single-stranded DNA generation was observed in the DMBA+vanadium–treated animals (Group C). Moreover, the native double-stranded DNA in Group C animals was almost twofold higher than in Group B animals. Table 4 shows the number of single-strand breaks per DNA fragment in the presence or absence of vanadium supplementation. There was a significant ($P < 0.001$) increase in the number after

**Table 3. Elemental Concentrations of Three Different Groups (µg/ml of blood)**

<table>
<thead>
<tr>
<th>Elements Detected</th>
<th>A (Normal)</th>
<th>B (DMBA Control)</th>
<th>C (DMBA + Vanadium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>370.5 ± 0.04a</td>
<td>577.5 ± 0.04</td>
<td>392.7 ± 0.03b</td>
</tr>
<tr>
<td>Cu</td>
<td>1.5 ± 0.06</td>
<td>0.89 ± 0.05</td>
<td>0.98 ± 0.06b</td>
</tr>
<tr>
<td>Zn</td>
<td>18.9 ± 0.01</td>
<td>9.0 ± 0.06</td>
<td>17.9 ± 0.02b</td>
</tr>
</tbody>
</table>

*a: Value represents mean ± SE of five rats.*

*b: Significantly different from Group B by Student’s $t$-test ($P < 0.01$).*

**Figure 3.** Effect of vanadium ($\text{NH}_4\text{VO}_3$, ammonium metavanadate) on the generation of DNA chain breaks in mammary gland of rats in the presence or absence of DMBA treatment. DSD (■), double-stranded DNA; SSD (□), single-stranded DNA. *$P < 0.001$ compared with Group A. *$P < 0.001$ compared with Group B.
DMBA treatment alone compared with control counterparts. Treatment with vanadium abated the generation of single-strand breaks to some extent. The protection offered by vanadium was 61% \((P < 0.001)\) (Table 4). Levels of single-strand breaks per DNA fragment among rats receiving only vanadium were no different from those of the normal controls.

### Cell Viability After Exposure to Vanadium

The cell viability of the mammary epithelial cells was assessed after exposure to 1.56–25 \(\mu\)M \(\text{NH}_4\text{VO}_3\) for 24 h (Fig. 4). The viability of the mammary epithelial cells was unaffected by 1.56–6.25 \(\mu\)M \(\text{NH}_4\text{VO}_3\) treatment for 24 h but decreased to 70% in response to 12.5 \(\mu\)M \(\text{NH}_4\text{VO}_3\). The viability of the cells further decreased as the concentration of \(\text{NH}_4\text{VO}_3\) was increased and was 14% with 25 \(\mu\)M \(\text{NH}_4\text{VO}_3\) exposure. Thus, it was evident that vanadium up to a dose of 6.25 \(\mu\)M was noncytotoxic in vitro.

![Figure 4. Effect of \(\text{NH}_4\text{VO}_3\) on cell viability. Mammary epithelial cells were treated with various concentrations of \(\text{NH}_4\text{VO}_3\) for 24 h. Cell viability was determined with the Procheck cell viability assay and expressed as percent of control. Results are mean ± SE of three independent experiments. *Significantly different from control at \(P < 0.05\).](image)

### Effect of Vanadium on Chromosomal Aberrations

In vitro studies using cells obtained from naive rats indicated that there was an increase in the numbers of CAs in mammary epithelial cells that received only DMBA compared with that in cells that received DMBA and vanadium (Table 5). Structural aberrations (individual plus exchange type) coupled with either numerical or physiological types were predominant in the group that only received DMBA. Numerical aberrations were represented by aneuploidy and polyploidy, whereas physiological aberrations were denoted by stickiness and pulverization. Vanadium at a dose of 6.25 \(\mu\)M markedly abated the incidence of CA, that is, vanadium provided 62.9% protection against the degree of CA noted in cells that received DMBA only. Treatment with DMBA alone evoked CAs that consisted of mainly structural aberrations, that is, gaps, breaks, and centromeric constriction (Fig. 5A). Major physiological CAs were sticky aneuploids (Fig. 5B) and sticky hyperploids (Fig. 5C), whereas major individual and exchange types were exemplified by gaps, fragments, and translocations (Fig. 5D) and centric fusions and rings (Fig. 5E).

### Discussion

This study emphasizes vanadium as a chemopreventive agent by its efficacy in combating DNA chain breaks and CAs on carcinogen exposure. CA is considered to be a good somatic marker as it occurs with greatest frequency in cells that are highly proliferative (32). CA is known to be an important somatic mutation, and it is clearly involved in the origin, progression, and diversification of certain cancers (33). Considerable biochemical, cytogenetic, molecular, and immunological evidence indicates that most neoplasms arise from a single altered cell with the progeny of that cell expanding as a neoplastic “clone” (34). This clonal evolution is a result of enhanced genetic instability within the tumor cell population (35). Our in vitro analysis of CAs demonstrates that vanadium suppresses both structural as well as numerical aberrations of chromosomes in cells exposed to DMBA.

A report suggests that metal-mediated oxidative DNA damage plays one of the important roles in chemical carcinogenesis (36). Vanadium shows additional protection by raising the level of zinc. Zinc is recognized to be important for stabilizing DNA, and it appears to reside longer in the nucleus than in any other cell compartment (36). It is known that, with the rise of intracellular levels of zinc, more iron will be displaced from the nucleoproteins and less OH– ion–driven DNA damage will occur (37). Mechanistically, zinc is involved in genetic stability and gene expression in a variety of ways, including structure of chromatin, replication of DNA, transcription of RNA as well as DNA repair (38). Iron is vital to life, being an important component of molecules undergoing redox reactions in the cells. This property also makes iron toxic because redox reactions may generate

### Table 4. Effect of Vanadium on the Number of Single-Strand Breaks (SSBs) per DNA Fragment in Rat Mammary Gland

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of SSB/DNA Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (normal)</td>
<td>0.07 ± 0.004(^b)</td>
</tr>
<tr>
<td>B (DMBA control)</td>
<td>1.22 ± 0.008(^c)</td>
</tr>
<tr>
<td>C (DMBA + vanadium)</td>
<td>0.47 ± 0.009(^d)</td>
</tr>
<tr>
<td>D (vanadium control without DMBA)</td>
<td>0.07 ± 0.005</td>
</tr>
</tbody>
</table>

\(a\): The percentage inhibition of the number of single-strand breaks per DNA fragment = \((1.22 – 0.47)/1.22 \times 100 = 61\%\).
\(b\): Values are mean ± SE of five animals.
\(c\): Significantly different from normal Group A by Student’s \(t\)-test \((P < 0.001)\).
\(d\): Significantly different from carcinogen control Group B by Student’s \(t\)-test \((P < 0.001)\).
ROS (39,40). ROS damages proteins, lipids, and DNA (41). Iron has also been shown to induce sister chromatid exchange and mediate H$_2$O$_2$-induced mutagenesis and cell transformation (42). Carcinogen control animals showed increases in intracellular levels of iron suggesting oxidative stress that leads to an increase in iron uptake (43–45). Supplementation with vanadium brought back the iron concentration to an almost-normal level, proving once again the chemopreventive efficacy of vanadium and its interactions with iron (46,47).

Copper in blood is bound to certain proteins such as ceruloplasmin, albumin, macroglobulins, transcupresin, and superoxide dismutase (SOD) (48). Cuproenzymes are important members of the antioxidant system of the organism (49). Oxidative stress is aggravated during the carcinogenic process and SOD is reduced (7). This leads to oxidative damage to the DNA. Vanadium supplementation has elevated the level of copper almost to its normal level. This may lead to the activation of cuproenzymes such as SOD and ceruloplasmin that combat with the oxidative stress and prevent the DNA damage.

**Table 5.** Effect of Vanadium on Chromosomal Aberrations in Mammary Epithelial Cells of Rats$^a$

<table>
<thead>
<tr>
<th>Groups</th>
<th>Structural Chromosomal Aberrations</th>
<th>Numerical Chromosomal Aberrations</th>
<th>Physiological Chromosomal Aberrations</th>
<th>Total Chromosomal Aberrations</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual Type</td>
<td>Exchange Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(chromatid breaks, fragments, gaps)</td>
<td>(centric fusion, translocation, rings)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumorigenic mammary epithelial cells without vanadium</td>
<td>55 22.00 50 20.00 47 18.80 56 22.40</td>
<td>18 7.20 17 6.80 22 8.80 77 30.8 ± 2.16</td>
<td>55 22.00 50 20.00 47 18.80 56 22.40</td>
<td>18 7.20 17 6.80 22 8.80 77 30.8 ± 2.16</td>
<td>62.9%</td>
</tr>
<tr>
<td>Tumorigenic mammary epithelial cells with vanadium</td>
<td>20 8.00 18 7.20 17 6.80 22 8.80 77 30.8 ± 2.16</td>
<td>55 22.00 50 20.00 47 18.80 56 22.40</td>
<td>18 7.20 17 6.80 22 8.80 77 30.8 ± 2.16</td>
<td>55 22.00 50 20.00 47 18.80 56 22.40</td>
<td>62.9%</td>
</tr>
</tbody>
</table>

$^a$: Percentage of chromosomal aberration was calculated on the basis of number of chromosomal aberrations per 250 cell plates.

$^b$: $P < 0.001$ significant from the other group.

**Figure 5.** A: A metaphase plate showing centric constriction (c) and a small fragment (f). B: Metaphase sticky aneuploid chromosomes. Note that the total chromosome number is less than 2n = 42. C: A sticky hyperploid metaphase showing abnormal chromosome number, that is, 2n = >42. D: Arrow indicates a small fragment, centromeric stretching coupled with a long arm translocation in a metaphase plate. E: A metaphase plate showing ring chromosome (r) and a small fragment (f).
Figure 5. (Continued)
Histology and morphometry were taken as end-point biomarkers of preneoplasia. Histological observations clearly showed that DMBA alone clearly damaged the normal architecture of rat mammary tissues. A typical epithelial hyperplasia with slight proliferation of mammary lobules, a preneoplastic condition, was observed. With vanadium supplementation, the tissue architecture was almost normal. Mild preneoplastic conditions were noted, proving that vanadium offered chemoprevention and/or protected against DMBA-induced damage. Morphometric measurements indicated that, with the vanadium cotreatment, both tumor incidence and multiplicity were reduced, and the mean latency period of tumor appearance was also delayed.

The cell viability result showed that the transformed epithelial cells were sensitive to the toxic higher doses of vanadium than to the low doses.

DNA strand breaks are generated from DNA base lesion induced by most chemical mutagens. DNA strand breaks also contribute to CAs (50). One hypothesis is that vanadium may promote excision repair activity by the restoration of metal ions (Zn, Cu, and Fe) levels. However, the exact mechanism of action of vanadium remains to be elucidated, and research in this mechanistic pathway is under progress in our laboratory.

The essentiality of vanadium in a multitude of biological and biochemical processes is evident. Vanadium thus may have immense potential for contributions to human benefit, particularly with supplementation of very low doses in the diet. Scientific explorations of further studies are needed to elucidate the defined role of vanadium at the physiological levels.

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