Vanadium : A Possible Role in the Protection of Host Cells Bearing Transplantable Murine Lymphoma

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ABSTRACT

The effect of oral administration of an optimum dose of ammonium monovanadate was studied in hosts bearing a transplantable ascitic lymphoma. This was supported by the biochemical indices relating to markers like reduced glutathione (GSH), extent of lipid peroxidation and activities of glutathione peroxidase (GPx) in hepatic tissues of the experimental animals. These biochemical parameters showed substantial alterations during the period of tumor progression in the experimental group treated with a low dose of vanadium (0.005 μM) suggesting a possible correlation between the observed variation in these markers and the survival rates in the animals receiving vanadium treatment.

Key words : Vanadium, Cancer chemoprevention, Survival.

INTRODUCTION

Vanadium is both a physiologically and pharmacologically active substance (13) detectable in air, water and soil (39). This, coupled with its occurence as a byproduct of petroleum and metallurgical refinings including the steel industry, has resulted in considerable work on vanadium toxicity. The toxicity of various vanadium compounds in animals, which varies depending on the route of administration, species and the nature of the compound used, has been reviewed in the past (21). In contradistinction to the proposed toxic properties of the element is the suggested regulatory role of vanadium (25, 3, 32) and its essentiality as a trace element in micro-organisms, plants and animal species (30).

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When cultures of malignant or benign mammalian cell lines were exposed to vanadium salts, stimulation as well as inhibition of cell growth or thymidine incorporation was observed (4, 34, 12, 22). It has been reported that vanadocene dichloride, an organometallo complex, has a cytotoxic action on Ehrlich Ascites Tumor cells *in vitro* and *in vivo* (16). We have also recently reported the biphasic role of ammonium monvanadate on a transplantable murine tumor model showing that inhibition, as well as promotion of cell proliferation by vanadium is a dose-dependent effect. An optimum dose of vanadium (0.005 μM) has been found by us to be effective in controlling cell proliferation of Dalton's ascites lymphoma in mice. Vanadium was found to increase the latency of tumor growth, control cell proliferation and prolong the survival of the hosts, with alterations in the activities of hepatic drug metabolizing enzymes (29).

GSH, the chief intra-cellular non-protein sulfhydryl compound involved in the protection of cells from free radical damge, an altered level of which has been observed in many experimental animal neoplasmes (27), has been known to be an important factor in determining the effectiveness of a variety of anticancer chemotherapeutic drugs (38). The extent of lipid peroxidation and activity of GPx system are also relevant in the study of neoplasms. Peroxidation of unsaturated lipids has been suspected of contributing to the process of cancer development (36). Slage *et al.* also reported the generation of active oxygen species which may lead to lipid peroxidation and formation of peroxides may be related to tumor promotion (33).

In view of these facts, the present study is an attempt to elucidate the role of vanadium at this optimum dose on glutathione related anti-oxidant defence system in the hepatic tissues of experimental aninals, inoculated with Dalton's lymphoma (DL) cells.

MATERIALS AND METHODS

Treatment of animals : Seven to eight week old inbred male Swiss albino mice, the average weight of each being 20±2 g, were housed in a temperature controlled (25.5±0.5℃) room with 50-60% humidity and were exposed to a 14 h cycle of light and darkness. The animals were divided into four groups, each consisting of twenty five mice. Three such sets of experiments were performed. All animals had access to pelleted mouse feed, supplied by Lipton India Ltd., Calcutta, and water *ad libitum*.

The four groups of mice were subjected to the study as follows:

Group I (Control) : Untreated normal mice
Group II (Lymphoma Control) : Mice bearing DL

Group III (0.005 μM vanadium control) : Mice treated with 0.005 μM of ammonium monovanadate/mouse/day

Group IV (Experimental) : DL bearing mice treated 0.005 μM of vanadium.

Groups III and IV were treated for seven days with the above mentioned dose of vanadium after which mice of Groups II and IV were acceptically inoculated intraperitoneally with DL by transplanting 2×10^6 tumor cells/0.2 m*l* saline into each mouse.

Biochemical Measurements

(a) *Reduced qlutathione (GSH) level*: The GSH levels in the hepatic tissue of the mice were determined by the method described by Anderson (2). The livers, after being perfused with normal saline, were rapidly removed, rinsed in water, blotted dry, and homogenized in 5 vol/g of wet tissue weight of 5% 5-sulfosalicylic acid. The tissue homogenate was centrifuged in 1.5 m*l* plastic vials in a microfuge for 5 mins. The supernatant solutions were stored at 4℃ until assayed. Daily buffer (NADPH 0.248 mg/m*l*) 5, 5'-dithio bis (2-nitrobenzoic acid) and water were pipetted in amounts described in the method into each cuvette. The cuvettes were warmed at 30℃ in a water bath for 12-15 mins. The sample was added with mixing to initiate the assay. Absorbance was measured at 412 nm. The amount of GSH was determined in μmol/g tissue from a standard curve in which the GSH equivalents were present.

(b) *Lipid peroxidation*: Lipid peroxidation of liver tissue of the mice was determined by the method given by Okhawa *et al.* (23) which is based on the formation of malondialdehyde (MDA). The organs were perfused with ice-cold 0.9% Nacl, tissue homogenates were prepared in a ratio of 1 g of wet tissue to 9 m*l* of 1.15% KCl. To samples less than 0.2 m*l* of 10% w/v tissue homogenate were added 0.2 m*l* of 8.1% sodium dodecyl sulfate, 1.5 m*l* of 20% acetic acid solution adjusted to pH 3.5 with NaOH and 1.5 m*l* of 8% aqueous solution of thiobarbituric acid. The mixture was made upto 4.0 m*l* with distilled water and heated in an oil bath at 95℃ for 60 min using a glass ball as a condenser. After cooling with tap water, 1.0 m*l* of distilled water and 5.0 m*l* of the mixture of n-butanol and pyridine (15 : 1 v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. 1, 1, 3, 3-tetramethoxypropane was used as an external standard and the level of

lipid peroxide was expressed as nmol of MDA.

(c) *Glutathione Peroxidase (GPx)*: The GPx activity was determined in the hepatic tissues by the method of Lawrence and Burk (18). Livers were perfused with 1.14 M NaCl and homogenised in 0.25 M sucrose. Supernatant fraction was prepared by centrifugation at 105000×g for 1 hr. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 E.U/m*l* GSSG-reductase, 1 mM GSH, 1.5 mM cumene hydroperoxide or 0.25 mM H_2O_2 in a total volume of 1 m*l*. All reagents except enzyme source and peroxide were combined. Enzyme source (0.1 m*l*) was added to 0.8 m*l* of the above mixture and allowed to incubate for 5 min at room temperature before initiation of the reaction by the addition of 0.1 m*l* of peroxide solution. Absorbance at 340 nm was recorded for 5 min and the activity was calculated from the slope of these lines as μmoles of NADPH oxidised/min. Protein was estimated by the method of Lowry *et al.* (26).

All the markers were monitered every 5th day throughout the period of study and were measured in the liver tissue because it is known that enzymes in the liver provide much more sensitive indicators of a distant neoplasm in the organism (10).

Statistical analysis: The results obtained were statistically analysed by using Student's 't' test.

RESULTS

The reduced glutathione level in the hepatic tissue of the control and experimental animals are depicted in Figure 1. The GPx activity and the extent of lipid peroxidation in the livers of the same are tabulated in Tables 1 and 2 respectively.

The hepatic GSH level in Group II animals maintained a normal level upto the 5th day and rose to a value as high as 10.56±1.32 μmol/g of hepatic tissue on the 15th day following tumor transplantation. Following this a steep decline to less than 1 μmol/g tissue was observed and death of the hosts occured around the 35th day.

While Group III animals showed slightly elevated GSH level throughout the period of study, the experimental animals of Group IV were found to maintain a sustained higher level of GSH that peaked on the 40th day with a value of 11.72±1.08 μmol/g tissue. The decline following this was steady and the animals concerned died around the 60th day.

As is evident from Table 1, the GPx activity started showing an increase

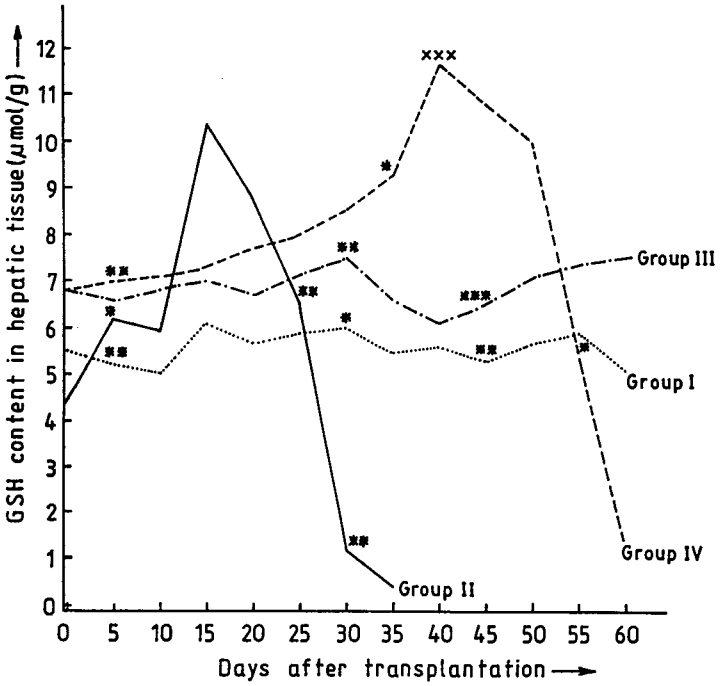

Fig. 1 Changes in the pattern of reduced glutathione levels in the hepatic tissues of vanadium treated experimental animals bearing DL against the control counterparts. Significant differences from control values at $p<.05$, $<.01$ and $<.001$ are represented as *, ** and *** respectively.

following the 5th day of tumor inoculation in Group II animals, peaking on the 15th day. The activity however declined after this to 12.03±0.98 n moles of NADPH oxidised/min/mg of protein on the 35th day.

In Group IV animals the GPx activity revealed the same increasing trend and maintained a sustained high activity level upto the 45th day. The decrease after this was abrupt reaching a value of 15.92±1.16 on the 60th day.

There was a steady but substantial increase in the extent of lipid peroxidation in the hepatic tissue following transplantation in Group II mice. The MDA content in the organ was maximum around the 20th day. The progressive decline after this continued till the last day of survival of the hosts.

In Group IV animals, the increase in MDA content was found to be considerably controlled in comparison to that of Group II animals upto about 20 days following transplantation. The increase following this peaked on the 45th day and decreased thereafter, showing a value as low as 6.01±2.40 n moles MDA/g of liver tissue around the 60th day.

Table 1 *Alteration in the GPx activity in hepatic tissues of DL bearing mice treated with vanadium and expressed as n mol of NADPH oxidised/min/mg of protein. Values are presented as means ± S. D. Significant differences from control values at p<.05, <.01 and <.001 are presented as *, ** and *** respectively.*

Days after Transplantation	Group I	Group II	Group III	Group IV
0	64.94±3.87	65.82±2.12	72.02±3.47	64.92± 3.81*
5	61.27±5.21	85.02±3.21*	74.35±3.21*	89.41± 3.21
10	63.95±3.21	120.12±6.82	76.21±4.18*	135.46± 5.86*
15	65.26±4.89	160.33±7.47**	75.89±2.72	175.22± 6.41**
20	61.01±2.66	112.45±5.21***	73.25±2.16	182.01± 8.28
25	62.94±2.88	93.95±3.17*	75.88±4.71	193.52± 7.21
30	59.18±5.02	32.83±1.22	74.91±3.22**	202.27±11.54**
35	58.26±2.17	12.03±0.98*	78.82±4.16*	215.92±15.61*
40	65.11±1.21		72.12±1.24***	250.85±25.22***
45	67.02±1.94		70.15±1.27	242.76±12.29
50	63.22±4.21		75.25±1.98*	141.72± 6.90
55	61.95±5.20		73.81±2.97	68.81± 3.21*
60	60.15±1.09		72.94±3.45**	15.92± 1.16**

Table 2 *Extent of lipid peroxidation in the hepatic tissues of vanadium treated mice inoculated with DL expressed in n moles of MDA/gm of tissue. Results are means ± S. D. *, ** and *** represent significant differences at p< .05, <.01 and <.001 respectively.*

Days after Transplantation	Group I	Group II	Group III	Group IV
0	9.98±0.87	9.29±1.35*	9.02±2.41*	8.42±0.71
5	7.50±1.21	27.81±3.41	10.5 ±0.87	8.91±1.05
10	9.45±1.05	76.12±7.24	11.26±1.24	19.26±2.31**
15	8.41±0.97	95.89±5.17*	11.91±1.07**	32.80±1.28
20	9.89±2.47	115.01±4.28	9.34±0.71	41.00±3.74*
25	9.45±3.46	74.66±4.27	9.86±1.78*	68.26±5.28***
30	7.64±1.07	28.15±1.31**	11.72±0.80	92.81±5.21
35	9.81±1.84	4.21±0.95	12.01±2.94	98.15±6.74
40	9.16±0.72		12.03±1.26	106.20±5.72
45	8.21±0.92		11.94±1.44*	110.70±4.89*
50	8.73±1.98		10.62±2.03	74.34±3.96*
55	7.94±2.03		10.14±0.98***	33.89±2.81
60	9.26±2.64		11.26±2.41	6.01±2.40**

DISCUSSION

GSH, a ubiquitous cellular constituent that serves a number of important biological functions (18, 3, 22, 23), is principally involved in the protective mechanisms against the deleterious actions of drugs and/or their metabolites (24) and heavy metal ions (31) by providing cells with a reducing environment, thus being effective in combating pathological conditions that are associated with formation of free radicals and reactive oxygen compounds (20).

The observed more or less normal GSH level in the hepatic tissues upto about 5 days following transplantation in DL bearing hosts, indicates, that there is little stress during the first phase of tumorigenesis and confirms the well known fact that hepatic GSH content is maintained at a relatively constant level by an autoregulatory system within a limited range of stress. Thereafter, the rise in the GSH level is possibly due to the resetting of the hosts defence mechanisms by which it strives to combat the pathogenicity of this tumorigenic process. The markedly elevated GSH level, which attains a peak on the 15th day following transplantation in the liver of the hosts with DL, could result from an attempt to prevent DNA damage which is known to occur in cancerous processes from oxidative stress (9) and that GSH can prevent DNA damage caused by certain free radicals has also been shown by Swauger *et al.* (35). In addition, increased GSH in tumors may afford protection from components of the immune system (27).

The differences in the lelels of GSH in the normal hepatic tissue and that of the hosts may reflect the overall proliferative status of the tissues. Allalunis-Turner *et al.* (1) recently demonstrated that proliferating cultures of both human and rodent tumor cells have higher GSH contents than do non-proliferating cultures. This view can be supported by yet another possibility related to the observation made by Kleinz-Szanto *et al.* (15) that in certain tumors there is an elevation of γ-glutamyl transpeptidase (GGT) activity. This corroborates with our recent observation on the tremendously increased GGT activity during the middle phase of DL (unpublished data). GGT can catalyze the degradation of extracellular GSH and may be involved in the production of precursors that can be used in the intracellular synthesis of GSH (27). Thus it is possible that elevated GSH contents of the tumors reflect increased GGT activities.

The depletion of GSH following this may be a consequence of the breakdown of the hosts defence mechanisms associated with inhibition of GSH synthesis.

In Group IV animals, GSH hepatic content is maintained at a steady high level that peaks on the 40th day, thereby enabling the host cells to maintain a stronger defence system for a prolonged time and is consistent with the survival

of the hosts upto approximately the 60th day, as has already been shown by us (29), establishing the beneficial effects of this optimum dose of vanadium treatment (0.005 μM).

The GPx system inhibits MDA formation in microsomal and mitochondrial preparations when the latter are subjected to conditions known to promote peroxidation of membrane lipids (6, 8). This enzyme system thus appears to play an important role in the protection of various cells from damage by bringing about the reduction of endogenously formed organic peroxides and hydroperoxides (19, 5) of polyunsaturated fatty acids present in the membranous portions of subcellular organelles.

GPx activity was found to achieve an increasing trend shortly after tumor inoculation in Group II animals, peaking on the 15th day, following transplantation, substantiating the fact the GPx is the first line of defence under conditions of oxidative stress (28). Following this maximal induction, the activity decreases due to the breakdown of regulatory control and associated loss of protein synthesis that occurs during the process of cell damage.

In vanadium treated Group IV animals, the GPx activity was found to maintain a 5 fold higher level upto the 45th day, suggesting a possible role of the studied dose of vanadium in controlling GPx enzyme system.

Lipid peroxidation is a natural phenomenon that is involved in peroxidative loss of unsaturated lipids, thus bringing about lipid degradation and membrane disordering (37). Peroxidised lipid has been considered to play a significant role in the pathogenesis of several diseases and may be taken as the molecular mechanism of cell injury under pathological conditions (17).

The persitant increase in the occurrance of lipid peroxidation following transplantation in Group II animals continues until the primary substrate becomes limiting proving the lethality of the cancerous process. The decrease in lipid peroxidation after the 20th day is possibly due to the change in lipid composition of membranes with a marked decrease in polyunsaturated fatty acids which are the main substrate for lipid peroxidation (7).

In Group IV mice however, the increase in lipid peroxidation occurs gradually. A drastic increase in the accumulation of MDA is suppressed to some extent by the triggered GPx system-which prevents the process of lipid peroxidation. The increase following the 20th day is abrupt, peaking on the 45th day with a value as high as 110.7 ± 4.89 n moles MDA/g of liver tissue and is followed by a decreasing trend. Thus treatment with vanadium seems to control the process of lipid peroxidation to a large extent by increasing the overall latency period.

The knowledge of the mechanism by which vanadium at this optimum dose

exerts its function at the cellular level in terms of the finger print of these parameters can give birth to the possibility of a new therapeutic agent for the future and is being undergone actively in our laboratory.

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