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Effects of Vitamin C and Vitamin A on Post Chromosomal Aberration *in vivo* Induced by Metanil Yellow and Zinc Chloride

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It is a well established fact that exposure to some physical and chemical agents can induce chromosomal aberrations in mammals (Bender *et al.* 1974, Giri *et al.* 1984a), and that some of these induced aberrations can persist for a long time (German 1979, Schull *et al.* 1982, Natarajan 1984). Although there is a strong correlation between the ability of an agent to induce chromosomal aberrations and its carcinogenicity (Purchase *et al.* 1978, Stich and Acton 1979, Cairns 1981, Yunis 1983) there is a long latent period between the appearance of the chromosomal damage and tumour expression of carcinogen in mammals. Ascorbic acid (vitamin C) is a strong reducing agent, and there is evidence to show that it has antimutagenic and anticlastogenic properties (Mirvish 1975, Prashad *et al.* 1978, Giri and Banerjee 1986). Similarly vitamin A is also known to exhibit such action (Bollag 1979, Lotan 1980, Stich *et al.* 1984). The two vitamins and synthetic retinoids were efficient inhibitors of carcinogenesis in several different animal models (Committee on Diet, Nutrition and Cancer, 1982).

It is interesting to note that most of these antimutagenic/anticlastogenic effects of vitamin C and vitamin A were detected using *in vitro* and *in vivo* short term tests by applying the vitamins concurrently with the chemicals (Rosin and Stich 1978, 1979, Rosin 1983). However, no reports are available on their activity on post-chromosomal damage. In this communication, we have explored the anticlastogenic activities of vitamin C and vitamin A after treatment with two well known clastogenic agents namely metanil yellow (Vaidya and Godbole 1978, Giri *et al.* 1984b) and zinc chloride (Giri and Banerjee 1986).

Materials and methods

A. *Acute Experiment on Sister Chromatid Exchange Studies*

Acute experiment on sister chromatid exchange was conducted to observe the role of these vitamins in reducing the toxic effect at an acute level. Twenty five laboratory bred Swiss albino male mice (*Mus musculus*) 90 to 100 days old, each weighing 30 gms were housed five to a cage (measuring 30 cm length × 22 cm breadth × 15 cm height) and acclimatized for 15 days prior to start of the experiment. The animals were obtained from the Cancer Research Institute, Parel, Bombay, India and maintained on standard commercial diet (Hindusthan Lever Ltd., India). Body weights were recorded twice a week. 20 mice were gavaged zinc chloride dissolved in distilled water at the dose of 330 mg/kg body weight at 12 noon.

While under anasthesia, 5 mice were implanted subcutaneously in the neck with a 50 mg tablet of 5-bromodeoxyuridine (5-BrDU) prior to zinc chloride treatment (Giri *et al.* 1986). Five mice served as control and were gavaged 0.05 ml of distilled water immediately after tablet implantation. After 22 hours the 10 BrDU implanted mice (5 zinc chloride gavaged

and 5 distilled water gavaged) were injected colchicine (at the dose of 5 mg/kg body weight) and were killed after 2 hours by cervical dislocation. Bone marrow preparations of chromosome were prepared accordingly (Giri *et al.* 1986). After 24 hours the remaining 3 sets of 15 zinc chloride treated mice were gavaged vitamin A (6.66 mg/kg body weight), vitamin C (16.66 mg/kg body weight) and distilled water (0.05 ml/animal) for 7 days (at 12 noon on each day). On the 8th day, each of 15 mice was implanted subcutaneously in the neck with a 50 mg tablet of 5-BrDU (Giri *et al.* 1986). On 9th day (at 10 A.M.) colchicine was injected i.p. to each mice at the rate of 5 mg/kg body weight. After 2 hours (at 12 noon) the animals were killed and bone marrow chromosomes were prepared accordingly (Giri *et al.* 1986).

Differential staining of the sister chromatids was carried out by a modification of the fluorescence-plus-Giemsa (FPG) technique (Perry and Wolff 1974). Slides, aged for 4–5 days, were stained for 10 min in Hoechst 33258 dissolved in NaCl/KCl solution; rinsed and mounted with M/15 Sørensen's phosphate buffer (pH 6.8). Then the slides were irradiated with a 254-nm UV mineralogic lamp for 30 min. Slides were incubated in 2×SSC (0.3 M NaCl, 0.03 M trisodium citrate) solution for 90 min at 59°C immediately after irradiation. Slides were then rinsed thoroughly in distilled water and stained with 7% phosphate-buffer-Giemsa solution for 10 min, and mounted as usual. 100 metaphase cells from 5 animals in each concentration in all the sets were scanned for SCEs.

The dose in the acute experiment was calculated based on the idea that zinc chloride administered over a period of 21 days in the chronic experiment, was administered by gavage as a single acute dosage. The dose of vitamin C and vitamin A selected was double of that administered in chronic treatment since the period here is 7 days only.

B. *Chronic Experiment on Bone Marrow Chromosomes*

Two sets of thirty five male mice of the same strain, each weighing 30 g were selected for bone marrow chromosome studies. Maintenance of the animals was same as described in the acute experiment. Metanil Yellow was dissolved in distilled water and was administered by gavage to 28 mice at a dose of 2 mg/kg body weight each day (at 12 noon) for 30 successive days. Seven mice served as control animals and were fed 0.05 ml of distilled water daily for 30 days. Seven treated mice and all the control mice were killed by cervical dislocation after the completion of 30 days. On the 31st day, seven treated mice were administered vitamin C (BDH, India—dissolved in distilled water) at a dose of 8.33 mg/kg body weight, seven treated mice were administered Retinyl palmitate (Sigma Chemicals, U.S.A.—dissolved in ground nut oil) at a dose of 3.33 mg/kg body weight and seven treated animals were administered 0.05 ml of distilled water. All the treatments were by gavage for another 30 days. These 21 mice were killed by cervical dislocation at the end of the treatment period. The dose of metanil yellow and zinc chloride was determined on the basis of earlier studies carried out by Giri and Banerjee (1986). The dose of vitamin C and vitamin A was selected on the basis of recommended doses for human i.e. 500 mg and 5000 I.U./day respectively.

Similarly zinc chloride was dissolved in distilled water and was administered by gavage to 28 mice at the dose 16.5 mg/kg body weight each day (at 12 noon) for 21 successive days. Seven mice served as control and were fed to 0.05 ml of distilled water for 21 days. Seven treated mice and all the control mice were killed by cervical dislocation after the completion of 21 days. On the 22nd day, the three sets of seven treated mice were administered vitamin C, vitamin A and distilled water respectively as in metanil yellow treated series for another 21 days. On the 43rd day all the 21 mice were killed by cervical dislocation.

For bone marrow chromosome studies, preparations were made following the usual colchicine-hypotonic flame drying Giemsa procedure (Sharma and Sharma 1980). A total of 600 metaphase plates were scanned from the seven animals sacrificed in each set of experi-

ment. Chromosomal aberrations were scanned at the metaphase stage. Chromatid and chromosomal breaks, chromatid and chromosomal exchanges and cells with more than 10 aberrations were scored (Sram 1975, Vargova *et al.* 1980). The number of breaks per metaphase (Z/B), was the fundamental index for comparison. In the Z/B category chromatid and chromosomal breaks, exchanges, dicentric chromosomes and cells with more than 10 aberrations were included. In this qualitative evaluation, the number of breaks were added in the following way: chromatid break—1 break, chromosomal break—1 break, chromosomal exchange—2 breaks, dicentric chromosome—2 breaks, cells with more than 10 aberrations—10 breaks (Vargova *et al.* 1980). Gaps were not included in view of the controversy regarding their significance (Bender *et al.* 1974, Savage 1975).

Results and discussion

Figure 1 shows the result on the induction of SCEs exposed to zinc chloride, zinc chloride and distilled water, zinc chloride and vitamin C and zinc chloride and vitamin A. Marked increase in the frequency of the SCEs was observed in all the treated series when compared with distilled water control. There was a significant decrease in the frequency of SCEs in the group treated with zinc chloride and vitamin C/vitamin A/distilled water when compared with the group treated zinc chloride alone for 24 hours. However, the frequency of SCE was still higher than that of the distilled water control for 24 hours. A significant decrease in the SCEs were observed in zinc chloride and vitamin C/vitamin A treated series when compared with that of zinc chloride and distilled water treated group. So vitamin C and vitamin A can reduce significantly the frequency of SCEs induced by zinc chloride. But there was no significant differences of SCEs between the two vitamin treated series. It has also been observed that neither the vitamins nor the distilled water can reduce the SCEs up to the distilled water control level. Thus the results on SCEs indicate that vitamin C and vitamin A can reduce the level of toxicity to a certain level when the damage has already taken place.

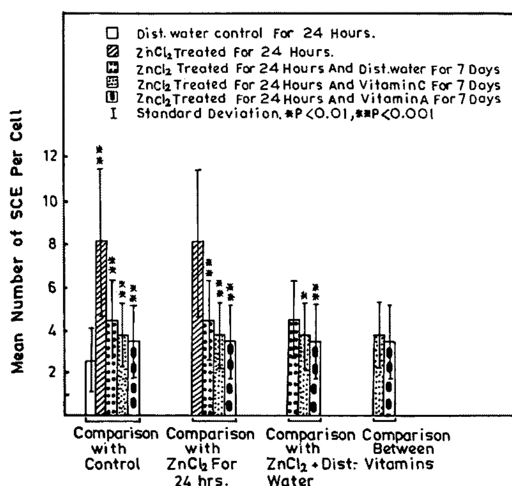

Fig. 1. Follow up of the sister chromatid exchanges induced by $ZnCl_2$ *in vivo* on mice.

A significant increase in the frequency of chromosomal aberrations (index Z/B) was found in the bone marrow of mice treated with metanil yellow and zinc chloride for 30 and 21 days respectively (Tables 1 and 2). There was significant decrease in the frequency of chromosomal aberrations in the bone marrow of mice treated with metanil yellow for 30 days and zinc chloride for 21 days and then subsequently treated with distilled water, vitamin C, vitamin A (retinyl palmitate) each separately for 30 and 21 days respectively when compared to Metanil Yellow and zinc chloride treated series. However, even at this sample time, the frequency of chromosomal aberration was still significantly higher than that observed following distilled water control; except in case of metanil yellow and vitamin C treated group (Table 3). No significant difference in chromosomal aberration was observed between metanil yellow with distilled water and metanil yellow with vitamin C/A. Similarly, no

significant difference was observed when zinc chloride with distilled water was compared to zinc chloride with vitamin C/A (Table 3).

Thus, the above results indicate that metanil yellow and zinc chloride were strongly clastogenic, which is in agreement with the observations of Vaidya and Godbole (1978), Giri *et al.* (1984b) and Giri and Banerjee (1986). It is interesting to note that the frequency of damaged cells induced by both the clastogens does not decline completely to the control levels either naturally or by treating it with vitamin C or vitamin A. Only in case of Metanil Yellow treated series vitamin C decreased the frequency of aberration to that of the distilled water control level. Thus, the antagonistic activity of both the vitamins cannot operate when the damage has already taken place. Again, when metanil yellow or zinc chloride with vitamin C treated group was compared to metanil yellow or zinc chloride with vitamin A, there was no significant difference in the aberrations. This indicates that the activity of vitamin C and A was more or less same in the repair of post chromosomal damage. Earlier report showed a significant decrease in the chromosomal aberrations when metanil yellow or zinc chloride was gavaged concurrently with vitamin C (Giri and Banerjee 1986).

Table 1. Follow up of the chromosomal aberrations induced by Metanil Yellow.

Substance	Dose (mg/kg)	No. of animals	n	Aberrant cells		Breaks (No.)	Cells with 10 abberra-tions	Z/B
				Number	%			
Distilled water	0	7	600	13	2.16	14	3	0.073±0.063
Metanil Yellow	2	7	600	75	12.50	40	40	0.734±0.149
Metanil Yellow + distilled water	2+0	7	600	23	3.83	18	10	0.192±0.116
Metanil Yellow + vitamin C	2+8.33	7	600	22	3.67	20	7	0.147±0.080
Metanil Yellow + vitamin A	2+3.33	7	600	23	4.00	22	8	0.169±0.079

n=Number of cells analysed.
Z/B=Number of aberrations per cell which is expressed as mean±S.D.

Table 2. Follow up of the chromosomal aberrations induced by zinc chloride.

Substance	Dose (mg/kg)	No. of animals	n	Aberrant cells		Breaks (No.)	Cell with 10 aberra-tions	Z/B
				Number	%			
Distilled water	0	7	600	11	1.83	11	3	0.068±0.057
Zinc chloride	16.50	7	600	93	15.50	41	58	1.041±0.186
Zinc chloride + distilled water	16.50 + 0	7	600	36	6.00	21	20	0.368±0.102
Zinc chloride + vitamin C	16.50 + 8.33	7	600	37	6.16	23	19	0.354±0.098
Zinc chloride + vitamin A	16.50 + 3.33	7	600	29	4.83	22	14	0.268±0.118

n=Number of cells analysed.
Z/B=Number of aberrations per eell which is expressed as mean±S.D.

The lower incidence of cancer among high consumers of vegetables and fruits has been attributed to the protective role of vitamin C or vitamin A (Stich and Rosin 1984). These conclusions were supported by the chemopreventive studies showing a regression of preneoplastic lesions following local or systemic administration of ascorbic acid (De Cosse *et al.* 1975, Bussey *et al.* 1982) or retinoids (Ryssel *et al.* 1971, Koch 1978, Bollag 1979, Gouveia *et al.* 1982). The anticarcinogens or antimutagens may exert their effect by trapping the ultimate carcinogens, acting as electron scavengers for free radicals or interfering with the activation of pre-carcinogens into their reactive electrophilic forms (Stich and Acton 1979, Stich *et al.* 1984). This phenomena possibly cannot operate in the present experiment, except in metanil yellow and vitamin C treated series, since the application of vitamins was carried after the induction of damage.

Table 3. Paired comparison by Mann-Whitney 'U' statistic.

Experiment with Metanil Yellow		Experiment with zinc chloride		Tabulated 'U'
Comparison with different groups	Calculated value of 'U'	Comparison with different groups	Calculated value of 'U'	
Distilled water control vs. Zinc chloride treatment	49*	Distilled water control vs. Metanil Yellow treatment	49*	U 0.0265 =9
Distilled water control vs. Zinc chloride+distilled water	49*	Distilled water control vs. Metanil Yellow+distilled water	45*	U 0.09735 =40
Distilled water control vs. Zinc chloride+vitamin C	49*	Distilled water control vs. Metanil Yellow+vitamin C	38	
Distilled water control vs. Zinc chloride+vitamin A	47*	Distilled water control vs. Metanil Yellow+vitamin A	41.5*	
Zinc chloride+distilled water vs. Zinc chloride+vitamin C	20.5	Metanil Yzllow+distilled water vs. Metanil Yellow+vitamin C	19.5	
Zinc chloride+distilled water vs. Zinc chloride+vitamin A	11	Metanil Yellow+distilled water vs. Metanil Yellow+vitamin A	25	
Zinc chloride+vitamin C vs. Zinc chloride+vitamin A	33	Zinc chloride+vitamin C vs. Zinc chloride+vitamin A	20	

* Significant at 5.63% level.

Although, the acute experiment on SCE and the chronic study of bone marrow chromosome gave different results but it is clear that the damage has not been fully repaired by the vitamins either in the acute or in the chronic experiment. Thus our observation may add a new dimension in the evaluation of the anticarcinogens or antimutagens using *in vivo* test systems.

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Summary

Effects of vitamin C and vitamin A were reported on the damaged bone marrow chromosomes and sister chromatid exchange studies on mice induced by both metanil yellow and zinc chioride. The results indicate that the damage is not repaired fully either naturally or by the prolonged treatment of vitamin A or vitamin C. Thus the vitamins cannot exhibit their antagonistic properties when the damage has already taken place.

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