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Protective role of vitamin c against cytotoxicity induced by adriamycin in mice

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Abstract

In the present investigation the protective effects of ascorbic acid in adriamycin induced cytotoxicity was evaluated in in vivo animal using analysis of chromosomal aberrations in somatic cells of mice. Three doses of ascorbic acid were selected for modulation and given to animals after priming with adriamycin. The animals were sacrificed after 24hr., 48 hr. and 72 hr and mitotic preparations were made according to the standard method and slides were prepared. In AA treated animals, the percentage of chromosomal aberrations were found to be in significant. But when perimed along with adriamycin, a significant decrease was observed in the percentage of chromosomal aberrations when animals primed with ascorbic acid. In conclusion our results indicate that vitamin C ameliorated genotoxicity induced by adrianycin in mice somatic in vivo.

Key-Words: Ascorbic Acid, Adriamycin, Genotoxicity

Introduction

A number of antineoplastic drugs are used to combat with different types of cancer which have shown to be mutagenic in various test systems. Various antineoplastic drugs such as cisplatin, cyclophosphamide, Tamoxifrn Genecitabine and Paclitaxel etc have shown to be clastogenic effects in various test systems[1-5].

Adramycin is one of most commonly used in malignant lymphomas, the drug is particularly beneficial in a wide range of pediatric and adult sarcomas. It has been show that chemotherapist agents including anthracyclins cause gene mutation, chromosomal aberrations rearrangents and aneuploids in somatic cells as well as an increased frequency of secondary treatment related tumor in human cancer survivors [6-8]. Further a significant increase was reported in patients in vivo in cytostatic treatment [9]. Because of the extensive and increasing use of adrianycin in successfully therapy regimes, an understanding of the mutagenic properties are important hence an attempt was made to study the potential mutagenic effect of adriemycin in mice system.

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Adriancycin, an anthracycline antibiotic is one of the most widely used anticancer drugs[10- 11] the main anticancer action of doxorubin is believed to involve DNA damage through inhalation of to poisomerase II. It causes generation of free radicals and the induction of oxidative stress associated with cellular injuries [12]. The free radical generation by adrianycin may participate as cardiotoxicity and genotoxicity in normal human cells[10-11] and in bone marrow cells of mice[13-14].

There is considerable evidence that the effects of mutagenic and carcinogenic agents can be altered by many dietary constituents. Vitamin C (VC) is an essential dietary nutrient required as a co-factor for many enzymes and a very efficient antioxidant, scavenging reactive oxygen and nitrogen species and protecting cells against free radical- mediated damage [15]. Besides exerting antioxidant influence directly, VC can promote the removal of oxidative DNA damage from the DNA and/or nucleotide pool, through the upregulation of repair enzymes [16]. The inhibitory effect of VC towards a number of mutagens/carcinogens was shown by many authors in humans and animals [17-19]. The goal of the present study is to evaluate the in vivo protective effect of VC against the DNA damage induced by adriamycin in mouse somatic and cells.

Material and methods

Eight-week-old healthy, laboratory bred Swiss albino mice (Mus musculus), weighing 25±3 g, were maintained under standard laboratory conditions at

temperature 22^0 C $\pm$ 2^0 C relative humidity 50 $\pm$ 10% and a 12-h photoperiod. Commercial pellet diet (Hindustan Lever, India) and deionised water were provided by libitum.

In the present studies on dose effect relationship, the animals injected intraperitoneally various doses of ADR (4,8,12 mg/kg). Ascorbic acid + ADR (5,10 and 20 + 4 mg/kg) and (5,10 and 20 + 8 mg/kg) and (5,10 and 20 + 12 mg/kg) respectively. Control groups of animals were maintained simultaneously, which received 0.1 ml saline and 0.1 ml mitomycin. The protocols were approved by instutional ethical committee of Osmania University, Hyderabad.

AA was given orally by gavage needle for 7 consecutive days. On the 7^{th} day, 1 hr after priming with (Ascorbic Acid) various split doses (10, 20, 40 mg/kg) was injected intraperitoneally to the animals for 4 consecutive days and the animals were killed 48 hrs after administration of the test chemical. The treatment for 48 hrs was kept to allow bone marrow cells to complete two cell cycles. The control and treated group of animals were sacrificed 6 hr after the last treatment by cervical dislocation. The bone marrow was flushed out into clean glass petridishes with hypotonic solution (0.75 M KCI) to get a fine homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37^0C for 45 minutes. Two slides for each were prepared from control and experimental animals. The staining was done within 24 hrs of the preparation according to the method of Preston *et al* [20]. The slides were screened for 100 well spread metaphases per animal was screened for the presence of various chromosomal aberrations like gaps, breaks, fragments, chromatid separations and polyploids in control and treated groups of animals. The data was analysed using the chi-square test.

Results and Conclusion

Various doses of ascorbic acid 5, 10, 20 mg/kg body weights were selected for the mutagenic effect of Adriamycin for 24, 48 and 72 hrs respectively and the observations were illustrated in table 4 at 24 hrs the frequencies were found to be higher then treated but the difference in the frequencies of chromosomal aberrations between controls and the ascorbic acid treated mice for 24 hrs, 48 and 72 hrs were analysed using X'-test and the results were found to be in significant (P>0.05)

The animals when treated with adriamycin showed a significant increase at all dose levels table 5 but when primed with 5, 10 and 20 mg/kg ascorbic acid along with the adriamycin the percentage of chromosomal aberration in somatic cells of mice showed a significant degrees at all dose levels. The difference in the frequency of chromosomal aberrations between the control and treated animals at 24, 48 and 72hrs treatment where subjected to statistical analysis and found to the significant table 4,5,6 and P<0.01 the details of the observations are given in the tables.

The results on the incidence of chromosomal aberrations in ascorbic acid treated mice are depited graphically in fig.1 were the data indicate a significant increase thus showing non mutagenic nature of ascorbic acid. Further the increased chromosomal aberrations in adriamycin treated animals were decreased stastically are showed in graphs fig. 2, 3 and 4 after priming with ascorbic acid.

The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound [20]. Chromosome aberrations observed in the present analysis were classified into structural numerical and other abnormalities these end points serve as indicators for evaluating the mutagenic potentials of test substances. Since there are considered as stable anomalies which continue to next generation. Further such variations in somatic tissues lead to malignancy.

Earlier analysis of chromosomal aberrations in somatic cells of mice were carried in our laboratory from last one decade with various drugs like asthalin Theophylline, Hevamizole Hetrazan, Pyrantal Paomate. Significant increase in the frequencies of chromosomal aberrations by 5-Fu was reported earlier [21]. The AA has been shown to the non-mutagenic in various plants and mice [22-24].

The protective effects of ascorbic and against 5-Flu has not reported earlier, this is first report showed the significant decrease in the incidence of chromosomal aberrations (pooled data) after primuis with AA. Hence the present data is compared with related studies. The results are in accordance with [25] who reported a regular dose of 10 mg/kg 1 day of Vit. C as a part of daily diet reduced T-2 toxin induced abnormalities in the somatic cells of mice. Further an antioxidant vitamin mixture (AVM) containing betacartone, alpha tocopherol ascorbic acid, rutin and micro elements zinc and selenium decreased the rate of chromosomal damage by x-ray induced micronuclei in the bone narrow polychromatic erythrocytes of mice [26]. In another study Sahu and Das [27] showed the reduced effects of Vit.C in Clofazimine (an leprosy drug) induced chromosomed aberrations in bone marrow cells of mice. Significant increase in the percentage of micronuclei in peripheral

blood cells of mice induced by bleomycin, an antineoplastic drug showed a reduction in response after the treatment of Vit.C Oral administration of organge drink Tampic containing Vit. C (8.9 mg/l) to FICBA x C 57 B16 mice has been shown to stabilize Crythrocyte membranes and reduce chromosomal aberrations in the murine bone manner cells induced by Cyclophosmide[28].

The present results are comparable with that of Giri [29] who reported the ascorbic acid showed protection against cisplatin induced chromosomal aberrations and micronuclei in bone marrow cells of mice. In another study Aly and Doniya (2002) studied the induction of chromosomal aberrations by Rifamcin (RMP) and found to be decreased significantly in mice treated with RMP + Vit. C.

Vit C acts as either a free radical scavenger or preoxidant producing hydrogen peroxide and free radicals [30-32]. As a physiological molecule ascorbic acid contributes to the natural antioxidant defense in cells. Ascorbic acid is considered to be most important antioxidant of plasma and at least in some cell types of intracellular has ascorbate may be in mM range [33].

Interest in the chemopreventive functions of antioxidants has grown considerably in recent years. Evidence accumulated over the years shows that people with high dietary intakes of fruits and vegetables are less likely to develop cancer than people who have low dietary intake of these foods. While many chemopreventives in fruits and vegetables may have anticancer properties, much interest has focused on vitamin C [34]. This study represents one of the premiere studies carried out to diminish the toxicity and the genotoxicity of the oxidative compound TMT by using the natural antioxidant compound VC. Vitamin C is a highly effective antioxidant. It acts as a reducing agent that can terminate free radical driven oxidation by being converted to a resonance-stabilized free radical. In this respect VC can protect indispensable molecules in the body, such as protein, lipids, carbohydrates and nucleic acids (DNA and RNA). VC also regenerates other antioxidants such as vitamin E [35]. Our results showed that concurrent administration of VC inhibited the DNA damage and chromosome aberrations induced by adriamycin in all tested doses. This ameliorative effect induced by VC may be resulted from enhancement of detoxification pathways that convert this reactive compound to less toxic and more easily excreted products [36] and/or through its action as the free radical scavenging efficiency. In addition, numerous in vitro and in vivo studies have evaluated the protective effects of VC against several radical generating chemicals [37-40].

In summary the modulating effect of ascorbic acid obtained in the present study warrant further investigation involving other test systems use of different protocols to evaluate the modulating effects of ascorbic acid against antineoplistic drugs, which are useful in cancer treatment are in progress.

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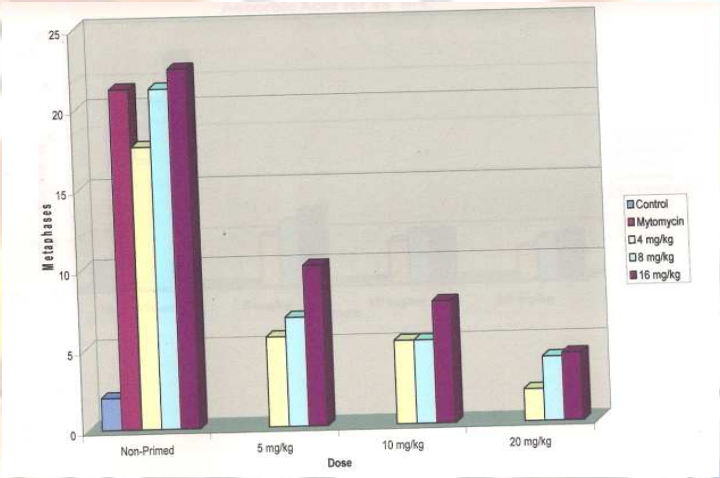

Fig. 2: Frequency of chromosomal aberrations recorded in somatic cells of mice treated with adriamycin and primed with ascorbic acid for 24 hrs.

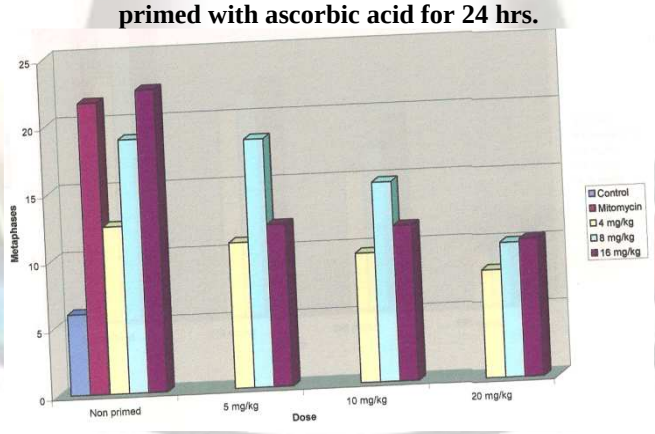

Fig. 4: Frequency of chromosomal aberrations recorded in somatic cells of mice treated with adriamycin and primed with ascorbic acid for 72 hrs.

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Table 1: Frequency of Chromosomal aberrations recorded in somatic cells of mice after treatment with various doses of Ascorbic Acid for 24, 48 and 72 hrs interval

Dose (mg/kg) and duration of treatment (hr)	24 hr		48 hr		72 hr	
	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)
Control	245	5	244	6	244	6
	(98.0)	(2.0)	(97.6)	(2.4)	(97.6)	(2.4)
4 mg/kg	244	6	243	7	243	7
	(97.6)	(2.4)*	(97.2)	(2.8)	(97.2)	(2.8)
8 mg/kg	243	7	243	7	241	9
	(97.2)	(2.8)*	(97.2)	(2.8)	(96.4)	(3.6)
16mg/kg	242	8	240	10	239	11
	(96.8)	(3.2)*	(96.0)	(4.0)	(95.6)	(4.4)

*P>0.05

Table 2: Frequency of Chromosomal aberrations recorded in somatic cells of mice treated with Adriamycin and Primed with Ascorbic Acid for 24 hrs treatment

Time Dose	Non primed		Primed with Ascorbic Acid					
			5.00 mg/kg		10.00 mg/kg		20.00 mg/kg	
	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored	Abnormal Metaphases Scored	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)
Control	245 (98.00)	5 (2.00)						
Mitomycin	218 (87.50)	32 (12.80)						
4 mg/kg	206 (82.40)	44 (17.60)	236 (94.40)	14 (5.60)	237 (94.80)	13 (5.20)	245 (98.00)	5 (2.00)
	197	53	233	17	237	13	240	10

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8 mg/kg	(78.50)	(21.20)	(93.20)	(6.80)	(94.80)	(5.20)	(96.00)	(4.00)
16mg/kg	194 (77.60)	56 (22.40)	225 (90.00)	25 (10.00)	231 (92.40)	19 (7.60)	238 (95.20)	12 (4.80)

The values in parentheses are percentages, *P<0.01

Table 3: Frequency of chromosomal aberrations recorded in somatic cells of mice treated with Adriamycin and primed with Ascorbic Acid for 48 hrs.

Time Dose	Non primed		Primed with Ascorbic Acid					
			5.00 mg/kg		10.00 mg/kg		20.00 mg/kg	
	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored	Abnormal Metaphases Scored	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)
Control	244 (97.60)	6 (2.40)						
Mitomycin	202 (80.80)	48 (19.20)						
4 mg/kg	220 (88.00)	30 (12.00)	238 (95.20)	12 (4.80)	240 (96.00)	10 (4.00)	242 (96.80)	8 (3.20)
8 mg/kg	205 (82.00)	45 (18.00)	235 (94.00)	15 (6.00)	238 (95.20)	12 (4.80)	240 (96.00)	10 (4.00)
16 mg/kg	194 (77.60)	56 (22.40)	232 (92.80)	18 (7.20)	238 (95.20)	12 (4.80)	239 (95.60)	11 (4.40)

The values in parentheses are percentages, *P<0.01

TABLE 4: Frequency of Chromosomal aberrations recorded in somatic cells of mice treated with Adriamycin and primed with Ascorbic Acid for 72 hrs treatment.

Time Dose	Non primed		Primed with Ascorbic Acid					
			5.00 mg/kg		10.00 mg/kg		20.00 mg/kg	
	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored	Abnormal Metaphases Scored	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)	Normal Metaphases Scored (%)	Abnormal Metaphases Scored (%)
Control	235 94.00	15 6.00						

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Mitomycin	196 78.40	54 21.60						
4 mg/kg	219 87.60	31 12.40	223 89.20	27 10.80	226 90.40	24 9.60	230 92.00	20 8.00
8 mg/kg	203 81.20	47 18.80	204 81.60	46 18.40	213 85.20	37 14.80	225 90.00	25 10.00
16mg/kg	194 77.60	56 22.40	220 88.00	30 12.00	221 88.50	29 11.50	224 89.80	26 10.20

The values in parentheses are percentages, * P<0.01