Chromosomal Aberrations and Sister Chromatid Exchanges in Cultured Human Lymphocytes III. Induced by Ascorbic Acid (Vitamin-C)¹

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Accepted June 6, 1991

The medicinal importance of ascorbic acid (vitamin C) was first demonstrated by James Lind in the mid-18th century when he found that juice of fresh citrus fruit could cure the scurvy. The human body does not synthesize ascorbic acid (Leevine 1986). It is abundantly present in fresh fruits and vegetables. Vitamin C deficient diet may lead to scurvy and impairment of immune response (Leevine 1986). Szent-Gyorgyi (1928) first isolated it in the pure form. It has been found that empirical formula is $C_6H_8O_6$ and chemically it is enolic form of 3-keto-L-gulofuranlactone:

Because of its broad pharmacological significances, it is widely used singly or in combination with other vitamins and drugs. Its mutagenic potentiality is controversial. Some of the workers have found it to be mutagenic (Stich *et al.* 1976, 1979, Gut-

tenplan 1977, Omura *et al.* 1978, Galloway and Painter 1979) while others have reported it to be non-mutagenic (Norkus *et al.* 1983). The present study was therefore undertaken to evaluate its genotoxic effects in cultured human lymphocytes system which will be helpful to determine its genetic hazards to human populations and consequently safe use.

Materials and methods

The mode of treatment has been described in Part I of this series. Solutions of different concentrations (0.1, 1.0, 10.0 and 100.0 $\mu\text{g/ml}$) were prepared in sterile deionized distilled water by serial dilution method.

Results

(A) Chromosomal aberrations

Aberrant metaphases did not show significant variations after both types of treatments (Tables 1, 2). Most common aberration was chromatid gap. Chromosome gap was observed occasionally (Treatment 'A', 1.0 $\mu\text{g/ml}$ —Table 1). Aberrant cells did not show multiple aberrations per cell.

(B) Sister chromatid exchanges (SCE's)

SCE's per cell were decreased significantly when cells were cultured in presence of 100.0 $\mu\text{g/ml}$ ascorbic acid (Table 3). Low dose treatment did not significantly alter SCE frequency

¹ C. D. R. I. Communication number 4803.

and cell cycle kinetics. Treatment 'B' did not show any effect on SCE frequency and cell cycle (Table 4).

Discussion

Ames *et al.* (1973) found ascorbic acid to be a weak mutagen. Later, Stich *et al.* (1976) found that the treatment of ascorbic acid and Cu^{2+} caused DNA fragmentation, DNA repair synthesis and chromosome aberrations in cultured human fibroblastic cells. SCE's were elevated in Chinese hamster cells and human lymphocytes exposed to A. A. (Macrae and Stich 1979, Galloway and Painter 1979). Rosin *et al.* (1980a) found ascorbic acid as a positive mutagen in hypoxanthine guanine phosphoribosyl transferase (HGPRT) gene locus mutation assay in cultured Chinese hamster ovary (CHO) cells. It has been suggested that in presence of O_2 and metal ions *e.g.* Cu^{+2} ascorbic acid is known to generate H_2O_2 and free radicals

Table 1. Chromosomal aberrations after treatment type 'A' of ascorbic acid

Treatment	No. of metaphases scored	Aberrant metaphases (%)	No. of aberration per aberrant cell	Type of aberrations (%)	
				Chromatid	Chromosome
				gap	gap
Control	100	1.00	1	1.0	0.0
0.1 $\mu\text{g/ml}$	100	1.00	1	1.0	0.0
1.0 "	100	2.00	1	1.0	1.0
10.0 "	100	0.0	0	0.0	0.0
100.0 "	100	1.00	1	1.0	0.0

Table 2. Chromosomal aberrations after treatment type 'B' of ascorbic acid

Treatment	No. of metaphases scored	Aberrant metaphases (%)	No. of aberration per aberrant cell	Type of aberrations (%)	
				Chromatid type	Chromosome type
				gap	gap
Control	100	0.0	0	0.0	0.0
0.1 $\mu\text{g/ml}$	100	1.0	1	1.0	0.0
1.0 "	100	0.0	0	0.0	0.0
10.0 "	100	2.0	1	2.0	0.0
100.0 "	100	2.0	1	2.0	0.0

derived from H_2O_2 (Weissberger and Luvalle 1944, Taqui Khan and Martell 1967) which can damage DNA and other cellular components (Fridovich 1976) and thereby causing mutation, chromosomal aberrations and sister chromatid exchanges (Stich *et al.* 1976, Morgan *et al.* 1976). This is further evident by the findings that catalase inhibits the magnitude of different biological end points (Morgan *et al.* 1976, Stich *et al.* 1978, Prasad *et al.* 1978, Rosin *et al.* 1980a). Several other transition metals (Mn^{+2} , Fe^{+2}) have also been found to enhance the mutagenicity of ascorbate (Stich *et al.* 1976, 1979). The above studies suggest that the interaction of ascorbic acid and metal ions generate H_2O_2 in the incubation mixture and that H_2O_2 or the radicals derived from H_2O_2 cause damage. Non-generation or scavenging of per oxide radicals dose not allow DNA damage. Contrary to the *in vitro* results, Speit *et al.* (1980) observed that treatment with extremely high doses (200–10,000 mg/kg b. wt.) of ascorbic acid had no effect on the SCE frequency and cell cycle kinetics in the bone marrow of the Chinese hamster. It has been suggested that this might because in contrast to *in vitro* conditions, the

Table 3. Sister chromatid exchanges after treatment Type 'A' of ascorbic acid

Table 3. Sister chromatid exchanges after treatment with 100.0 µg/ml of 5-fluorouracil												
Treatment	No. of metaphases scored	SCE/meta-phase (Mean±SD)	SCE range	% metaphases with different SCE ranges						Cell Cycle kinetic (%)		
				0-3	4-6	7-9	10-12	13-16	>16	1st Cycle	2nd Cycle	3rd Cycle
Control	25	10.08±3.54	3-18	4.0	4.0	40.0	28.0	16.0	8.0	19.69	33.33	46.96
0.1 µg/ml	25	10.48±3.92	6-22	0.0	12.0	36.0	20.0	24.0	8.0	22.53	39.43	38.02
1.0 "	25	9.96±2.58	6-17	0.0	8.0	48.0	28.0	12.0	4.0	31.70	31.70	36.58
10.0 "	25	10.16±3.47	5-19	0.0	12.0	32.0	40.0	4.0	4.0	23.52	38.23	38.23
100.0 "	25	8.08±2.9*	5-13	4.0	28.0	44.0	16.0	8.0	0.0	23.52	41.17	35.29

* Significant from control at 0.05 level t-test.

Table 4. Sister chromatid exchanges after treatment Type 'B' of ascorbic acid

Treatment	No. of metaphases scored	SCE/meta-phases (Mean $\pm$ SD)	SCE range	% metaphases with different SCE ranges					Cell Cycle kinetic (%)			
				0-3	4-6	7-9	10-12	13-16	>16	1st Cycle	2nd Cycle	3rd Cycle
Control	25	10.0 $\pm$ 4.18	3-16	8.0	4.0	44.0	20.0	20.0	4.0	21.42	16.66	61.90
0.1 μ g/ml	25	9.0 $\pm$ 3.76	3-16	8.0	16.0	16.0	20.0	12.0	0.0	14.28	21.42	64.28
1.0 "	25	11.68 $\pm$ 3.42	6-20	0.0	4.0	24.0	24.0	32.0	16.0	11.53	19.23	69.23
10.0 "	25	9.6 $\pm$ 3.20	5-15	0.0	24.0	24.0	32.0	20.0	0.0	10.5	21.05	68.42
100.0 "	25	10.28 $\pm$ 2.92	6-17	0.0	4.0	48.0	24.0	20.0	4.0	17.14	14.28	68.57

cells in whole mammalian organisms seems well protected against damage by H_2O_2 and radicals, since glutathione per oxidase, catalase and super oxide dismutase prevent the destructive action of per oxides and radicals. Thus it seems that if an organism is deficient of these factors, ascorbic acid may cause genetic damage.

In our study the lack of chromosomal aberrations and SCE inducing effect seems due to absence of metal ions because culture medium was prepared in deionized sterile distilled water. Further, significant lowering of SCE's after continuous treatment of 100.0 $\mu\text{g/ml}$ ascorbic acid (Table 3) suggests that in absence of metal ions it might have some kind of DNA repair inducing effects resulting in decreased spontaneous SCE frequency. Brief treatment (type 'B' of 3 hr) did not appear sufficient to induce these repair process (es). Its DNA repair inducing property is also evident by other *in vitro* studies (Benedict *et al.* 1980, Gol Winkler *et al.* 1980, Rosin *et al.* 1980b) and *in vivo* studies (Cameron and Paulling 1978, Sram *et al.* 1983). Benedict *et al.* (1980), Gol-Winkler *et al.* (1980) and Rosin *et al.* (1980b) found that ascorbic acid suppressed the transformation of C3H/10T 1/2 cells induced by 3 methyl chol-anthrene. Cameron and Paulling (1978) noticed that cancer patients treated with ascorbate were found to have a mean survival time of 300 days longer than that of the control. Sram *et al.* (1983) observed that daily administration of 1.0 g ascorbic acid to coal tar workers significantly decreased the aberrant metaphases. Thus it is evident that ascorbic acid is potent to induce some kind of DNA repair process (es).

From the present study it can be concluded that ascorbic acid itself is not DNA damaging agent. It can show mutagenic and genotoxic activities in presence of metal ions. In absence of metal ions it appears rather DNA repair inducer and enhancer of life expectancy. Our this view gets strengthened by the study of Norkus *et al.* (1983). In a very sensitive host mediated mutagenicity assay they found that administration of 5000 mg/kg to guinea pigs did not show mutagenic response to *S. typhimurium* TA 100. No significant effect on SCE frequency was noticed by Speit *et al.* (1980) at very high dose (10,000 mg/kg b. wt.) in bone marrow cells of Chinese hamster. However, the study of Stich *et al.* (1980) warns and suggests that combinational preparations of ascorbic acid with iron have clastogenic effects. Therefore, it is stressed to have detailed interaction studies with other drugs so that its toxic effect could be eliminated.

Summary

Ascorbic acid (A. A.) (vitamin C) is one of the most widely used (singly or in combinations) vitamins. Because of its broad pharmacological significances and presence in natural food products, the present study was carried out to evaluate its cytogenetic effects in *in vitro* human lymphocytes. Concentrations screened were 0.1, 1.0, 10.0 and 100.0 $\mu\text{g/ml}$. Aberrant metaphases did not show significant variation after both types of treatments. 3 hr treatment in G_2 /early S phase did not affect the SCE frequency, while continuous treatment (100.0 $\mu\text{g/ml}$) significantly reduced the same. This suggested that constant presence of A. A. might have potentiality to induce DNA repair. Mutagenic and antimutagenic effects of A. A. have been discussed in detail. From the present study it seems that A. A. itself is not mutagenic but may have mutagenic action in presence of metal ions (e. g. Fe^{+2}).

Acknowledgement

Thanks are due to Council of Scientific and Industrial Research, New Delhi, India for providing financial assistance.

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