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Anticlastogenic action of vitamin C against genotoxicity of estrogenic drug diethylstilbestrol (DES) in the human lymphocyte chromosomes

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Abstract: *Several sex steroids and estrogenic drugs are genotoxic in varying conditions and cause oxidative stress, which has been a field of interest to study the molecular mechanism of the genetic damage. Among the estrogenic drugs, a strong toxic effect is exerted by diethylstilbestrol (DES). In the present study it has been attempted to study its genotoxic effects in human lymphocyte assay system along with ameliorative or anticlastogenic effects of vitamin C. The drug was used with different dosage of concentrations on human lymphocytes administered in vitro. The parameters used were Sister Chromatid Exchanges (SCEs) and Chromosomal Aberrations (CAs). Higher levels of clastogeny and SCEs have been observed indicating significant damaging effect by the drug. Interesting ameliorating effects were observed in the presence of vitamin C which is a well-known antioxidant. The results support the possibility of practical application of natural protectors against the mutagenic / genotoxic action of chemical mutagens.*

Key words: *Anticlastogenic, Genotoxicity, Human lymphocytes, Vitamin C, Diethylstilbestrol.*

Introduction

It is well known that natural and synthetically produced hormonal drugs occupy an important place in the therapy of a whole series of endocrinal, gynaecological and internal diseases (Bali *et al.,* 1990; Dhillon *et al.,* 1994; Hundal *et al.,* 1997; Shadab *et al.,* 1999). Synthetic sex steroidal drugs are widely used as oral contraceptives in addition to their use in the treatment of various menstrual disorders, different types of cancer and in hormonal replacement therapy (Ahmad *et al.*, 2000b, 2001). Several estrogens (Ahmad *et al.,* 2000a, 2003) and other synthetic steroids (Shadab *et al.,* 2002, Ahmad *et al*., 2002) have been shown to possess genotoxic potentials. The synthetic estrogens and progestins have been evaluated to show their genotoxic effects (Ahmad *et al.,* 2001, 2003). It has been established, on the basis of clinical and experimental data, that they play a decisive role in the origin of cancer of the mammary glands and reproductive organs in the humans and animals (Ulfelder, 1976; Bishun and Williams, 1977). Among the synthetic estrogen preparations, a strong toxic effect is exerted by the diethylstilbestrol (DES), which exhibits transplacental carcinogenic and teratogenic effects (Folkman, 1971). The available literature on the genetic activity of DES in various test systems is contradictory. DES does not produce mutations in *Salmonella typhimurium* (Glatt *et al*., 1979), although DES induces sister chromatid exchanges (SCEs) in human fibroblasts and lymphocytes (Hill and Wolff, 1983). DES also delays cell proliferation in human lymphocytes. It is therefore, desirable to search for substances that may succeed appreciably minimizing or nullifying the carcinogenic or genotoxic property of this drug. Such substances have been given the common name of bio-antimutagens (Kada, 1984). L-ascorbic acid (vitamin C) is one such compound, which has been reported to have antioxidant properties (Gebhart *et al*., 1985; Ghaskabdi and Vaidya, 1989). It has been found that vitamin C (VC) is effective protector against the detrimental effects of cytostatics (Wang *et al*., 1980) minimises mitoinhibition and clastogenecity (Hoda and Sinha, 1993). Therefore, the present work was taken to explore the anti-clastogenic and anti SCE effects of VC against the genotoxic effect of DES in human lymphocytes.

Materials and Methods

The human lymphocyte culturing was done following the technique of Moorhead *et al*. (1960) for 72 hr, with suitable modifications for setting 5ml of culture medium (RPMI-1640: containing 10% foetal calf serum, phytohaemagglutinin and antibiotics). A DES powder was dissolved in dimethylsulphoxide (DMSO) and introduced into the culture after it was set up in a dose of 1.3×10^{-5} M. This optimal was found to be effective in producing chromosomal aberrations and SCEs. To obtain differential staining of the sister chromatids, 5' bromodeoxyuridine (5'BrdU) was added to the medium at a concentration of 3μg/ml and incubated in the dark. The slides were prepared by air-drying method. The differential staining of the chromatids was achieved as per Wolff and Perry (1974) and Azfer and Afzal (1996).

The following final concentrations of the vitamin C were used. VCI: $5xl0^{-6}$ M, VCII: 10^{-5} M and VCIII: $5xIO^{-5}$ M. DES and vitamins were added simultaneously for different durations (i.e. 24, 48 and 72 hr). As far as possible, 300 and 50 well spread metaphases per concentration were analyzed microscopically for structural chromosomal aberrations and SCEs, respectively.

Results and Discussion

The drug (DES) caused time dependent manifold increase in the incidence of individual as well as gross anomalies in the metaphase chromosomes as compared to the

Table – 1: The anticlastogenic effects of VC on the chromosome damaging action of DES (N=300 cells).

Treatment	Duration 'h'	Types of aberrations		Total ±SE	% CMA
		Chromatid	Chromosome		
Control	72	2.33	1.00	3.33±1.04	2.00±0.81
VCI($5X10^{-6}$)	72	2.67	1.33	4.00±1.13	3.33±1.04
VCII(10^{-5})	72	3.00	1.67	4.67±1.22	4.00±1.13
VCIII($5X10^{-5}$)	72	3.00	2.00	5.00±1.26	4.00±1.13
DES	24	6.66	3.33	9.99±1.73*	5.66±1.33
	48	8.33	6.00	14.33±2.02*	11.66±1.85
	72	11.66	7.33	18.99±2.31*	14.00±2.00
VCI+DES	24	4.33	1.00	5.33±130	5.00±1.26
	48	4.00	1.66	5.66±1.33	4.66±1.22
	72	3.33	0.00	3.33±1.04	1.33±0.66
VCII+DES	24	3.66	1.33	4.99±1.26	4.00±1.33
	48	2.66	1.66	4.32±1.17	4.00±1.33
	72	2.66	1.00	3.66±1.08	2.66±0.93
VCIII+DES	24	3.00	1.00	4.00±1.13	3.35±1.04
	48	5.33	1.66	6.99±1.47*	4.00±1.13
	72	6.66	2.66	9.32±1.68*	6.00±1.37

VC: Vitamin C; DES: Diethylstilbestrol; SE: Standard error; CMA: Cell with multiple aberration *: Significance at P<0.05.

Table – 2: Influence of vitamin C on the frequency of SCE induced by DES in the human lymphocytes *in vitro.*

Treatment	No. of cells	Total SCE	SCE/Cell	
			Range	Average (± SE)
Control	50	116	0-6	2.32±0.17
VC	50	110	0-4	2.20±0.14
DES	50	450	4-20	8.50±1.61*
DES+VCI ($5x10^{-6}$M)	50	123	0-6	2.46±0.19
DES+VCII (10^{-5}M)	50	125	1-8	2.50±0.20
DES+VCIII ($5x10^{-5}$M)	50	152	2-10	3.04±0.52

SCE: Sister chromatid exchange; *: Significance at p<0.05

normal control (Table 1). At 72 hr the drug induced highest aberrations. The VC alone produced only a marginal increase in the frequency of aberrations, which were never significant. Thus VC does not seem to be clastogenic. Significant decrease in the incidence of chromosomal aberrations was noticed when DES and VC (VC+DES) were given concurrently. At 72 hr the combined doses of DES and VCI & VCII produced many fold decrease in the incidence of abnormal metaphases while VCIII (V III + DES) could produce an increased rate of aberrations.

Comparable anti-clastogenic effects of vitamin on the chromosome- damaging action of carcinogens were reported by Shamberger *et al*. (1979) from human lymphocyte cultures. Their protective action was also found if ionizing radiations were the inducer of chromosome damage in onion root tips (Barthelmes *et al*., 1968). Gebhart *et al*. (1984) reviewed in detail a series of reports on antimutagenic action of VC on the effect of nitro and nitroso compounds, metal ions, and dietary components in bacteria. Dion *et al*. (1982) found a decreasing effect of a dietary application (400 mg/ml) of vitamins C and E on fecal mutagencity of normal donors, after Bruce *et al*. (1977), had shown the same for VC. It has been found that VC can minimise mitoinhibition (Hoda and Sinha, 1985) and anticlastogenicity (Sinha and Bose, 1989) in the bone- marrow cells of mice and it could even cure cancerous neurons of glioma cells in culture produced by methyl mercuric chloride (Prasad and Ramanujam, 1980).

The results suggest that inhibition of genotoxicity by vitamin C may be produced by way of (i) scavenging of the toxic molecules from blood cells, (ii) detoxification or incapacitation of the drug molecules via chemical processes, (iii) hindrance in the formation of DNA-DES adducts, especially with guanine bases and (iv) elimination of abnormality-bearing cells by their apoptosis (Thompson, 1994).

DES caused a highly significant increase of SCEs (8.50±1.61) than the mean SCE value of 2.3±0.17 observed in the normal control (Table 2). The concurrent administration of VCI and VCII with DES decreased the mean frequency of SCE, while VCIII could not minimize the SCE frequency.

The distinct differences in the anti-clastogenic action on the generation of chromatid and chromosome breaks by the clastogen were noted. Considering the relatively shorter time of exposure of the unsynchronized cell population, this observation indicated a rather specific action of the anticlastogen (VC) on

the aberrations produced by DES. Certainly, different chromatid/chromosome aberrations have different relations to cell-cycle transit and their peak frequencies can occur at different times (Gebhart *et al.,* 1984). The incidence of individual types of chromosomal anomalies and cells with multiple aberrations (CMA) induced by the drug was almost equal. When VC plus DES were administered for shorter duration (24 and 48 hr) the incidence of two types of aberrations decreased in the same manner as above. The reverse picture was obtained (% CMA decreased more than the individual chromosome damage) when the combination of DES and vitamins were administered. It may, therefore, be suggested that VC may promote or allow a repair system as it leads to an enhanced rate of apoptotic elimination of such damaged cells followed by compensatory division as cell replacement repair processes among the remaining normal and resistant cells. This might have been so because every organ/tissue must have in it an optimum number of healthy functioning cells (Leblond, 1972).

The decreasing action of VC on the SCE frequency induced by DES is evident. Our study is in line with the study conducted by Galloway and Painter (1979) and Spiet *et al.* (1980). They also pointed out that the VC at higher concentration has been shown to increase SCE frequencies. The precise mechanism of VC against the toxic effects of DES is still not known. However, it can be said that DES molecules which cause DNA break (Hill and Wolff, 1983) and block its replication, may be prevented by the use of vitamin which produce the defensive effect, as observed in the present case.

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