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# Induction of Chromosomal Aberrations and Sister Chromatid Exchanges by Cyproterone Acetate in Human Lymphocytes

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**ABSTRACT** The genotoxicity study of cyproterone acetate used as antiandrogen was carried out on human lymphocyte chromosomes using chromosomal aberrations and sister chromatid exchanges as parameters. The study was carried out in the presence as well as absence of metabolic activation (S9 mix). The effect of cyproterone acetate was studied as 5, 10, 20, 30 μM and was found to be genotoxic at 20 and 30 μM both in the presence as well as absence of metabolic activation system. The results suggest a genotoxic and cytotoxic effect of cyproterone acetate in human peripheral blood cultures *in vitro*.

## INTRODUCTION

Cyproterone acetate is a potent steroidal antiandrogen with progestational activity. It is used alone or in combination with ethinyl estradiol or estradiol valerate in the treatment of women suffering from disorders associated with androgenization, e.g. acne or hirsuitism. Cyproterone acetate competes with dihydrotestosterone for the androgen receptors and inhibits translocation of the hormone receptor complex into the cell nucleus (Sciarra et al. 1990). Cyproterone acetate has been shown to induce DNA repair synthesis in rat and human hepatocytes (Topinka et al. 1995; Neumann et al. 1992; Martelli et al. 1995; Kasper and Müller 1996) and to form adducts in rat liver cells (Topinka et al. 1996). In female rats, DNA adducts have been observed at low doses of cyproterone acetate, which are in the range of the therapeutic doses used in women (Werner et al. 1995). Further findings shows that cyproterone acetate is not only genotoxic but also is a tumour initiating agent in the liver of female rats (Deml et al. 1993; Martelli et al. 1996). Since there are no reports on the evaluation of genotoxic potential of cyproterone acetate using chromosomal aberrations and sister chromatid exchanges as a parameter, the study reported herein was conducted to evaluated genotoxic effects of cyproterone acetate on human lymphocytes *in vitro* in the presence as well as absence of metabolic activation system.

## MATERIAL AND METHODS

Duplicate peripheral blood cultures were prepared according to Carballo et al. (1993). Briefly about 0.5 ml heparinized blood samples were obtained from two adult healthy female donors and were placed in a sterile flask containing 7 ml of RPMI 1640 (Gibco) medium supplemented with 1.5 ml fetal calf serum(Gibco), 0.1 ml phytohaemagglutinin (Gibco) and 0.1 ml antibiotic-antimycotic mixture (Gibco) and was kept for 24 hr at 37°C in an incubator.

For chromosomal aberrations analysis, cyproterone acetate at a final concentration of 5, 10, 20 and 30 μM dissolved in dimethylsulphoxide (Merck, India) was added to the culture flask after 24 hr of the initiation of the culture and kept for another 48 hr. Dimethylsulphoxide (5 μl/ml) and Mitomycin-C (Sigma) served as negative control and positive control respectively. For metabolic activation experiments 0.8 ml of S9 mix was given along with the cyproterone acetate treatments. Liver S9 fraction (S9 mix) was prepared as per standard procedures of Maron and Ames (1983). Swiss albino healthy rats (Wistar Strains) each weighing about 200 g was given 0.1% phenobarbitone (Sigma) in drinking water for 1 week, for the induction of liver enzymatic activities. After 1 week rats were sacrificed and livers were obtained immediately, homogenized and centrifuged at 9000 rpm in 0.15M KCl. Cyclophosphamide (Sigma) served as a positive

control for metabolic activation experiments. One hour prior to harvesting 0.2 ml of colchicine (0.2 µg/ml, Microlab) was added to the culture flasks. Cells were centrifuged at 1000 rpm for 10 min. The supernatant was removed and 5 ml of pre-warmed (37°C) 0.075 M KCl hypotonic solution was added. Cells were resuspended and incubated at 37°C for 15 minutes. The supernatant was removed by centrifugation, and 5 ml of fixative (methanol: glacial acetic acid; 3:1) was added. The fixative was removed by centrifugation and the process was repeated twice. Slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 mins. At least 300 metaphases were examined for the occurrence of different types of chromosome breakage frequencies. Chromatid exchange configurations and dicentric chromosomes were scored as two (2) breaks. The criteria to classify the different types of aberrations were in accordance with the recommendations of EHC 46 for Environmental Monitoring of Human Population (IPCS 1985).

For sister chromatid exchange analysis, bromodeoxyuridine (BrdU, 10 µg/ml, Sigma) was added at the beginning of the culture. After 24 hr of the initiation of culture the treatments for both experiments i.e. presence and absence of metabolic activation system (S9 mix) were given same as described in chromosomal aberrations analysis. One hour prior to harvesting, 0.2 ml of colchicine (0.2 µg/ml) was given. Hypotonic treatment and fixation was done in the same way as described for chromosomal aberrations analysis. The slides were processed according to Perry and Wolff (1974) with some modifications. Slides were stained for 20 min in a 0.05% (w/v) Hoechst 33258 solution rinsed with tap water and placed under a UV lamp for 90 min, covered with sorensen's buffer pH 6.8, and stained with 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 mins. The sister chromatid exchange average was taken from an analysis of the metaphases during the second cycle of divisions.

The means of frequencies of sister chromatid exchange were statistically analysed by student's 't' test and the values of abnormal cells were analysed by the chi-square test. The level of significance was tested from standard statistical tables of Fisher and Yates (1963).

## RESULTS AND DISCUSSION

Cyproterone acetate increases the number of abnormal cells at 20 and 30 µM both in the presence as well as absence of metabolic activation system (Table 1 and 2). The aberrations observed were mostly chromatid and chromosome breaks. Chromatid exchanges were also seen at 20 and 30 µM both in the absence as well as presence of metabolic activation system (S9 mix).

In sister chromatid exchanges analysis a clear dose dependent increase in sister chromatid exchanges/cell was observed both in the absence as well as presence of metabolic activation system (Table 3). A significant increase was observed at

**Table 1: Structural chromosomal aberrations (CAs) in human lymphocytes treated with cyproterone acetate without S9 mix.**

| *Treatment* | *Cells scored* | *Abnormal cells (%)* | *Total structural CA* | | | | | *Total breaks without gaps (%)* |
|---|---|---|---|---|---|---|---|---|
| | | | *Gaps* | *CTB* | *CSB* | *CTE* | *DIC* | |
| *Cyproterone acetate* | | | | | | | | |
| 5 µM | 300 | 10 (3.34) | 8 | 13 | 2 | - | - | 15 (5.00) |
| 10 µM | 300 | 12 (4.00) | 10 | 18 | 3 | - | - | 21 (7.00) |
| 20 µM | 300 | 34 (11.34)* | 31 | 40 | 14 | 4 | - | 62 (20.67) |
| 30 µM | 300 | 39 (13.00)* | 37 | 47 | 19 | 6 | - | 78 (26.00) |
| Untreated | 300 | 5 (1.67) | 4 | 2 | 1 | - | - | 3 (1.00) |
| *Negative control* | | | | | | | | |
| DMSO (5 µl/ml) | 300 | 6 (2.00) | 5 | 3 | 1 | - | - | 4 (1.34) |
| *Positive control* | | | | | | | | |
| Mitomycin C (0.3 µg/ml) | 300 | 140 (46.67)** | 48 | 53 | 29 | 5 | 2 | 96 (32.00) |

Significant at *P < 0.005, ** P < 0.001 Vs Untreated (chi-square test)

DMSO: Dimethylsulphoxide; CTB: Chromatid break; CSB: Chromosome break; CTE: Chromatid exchange; DIC: Dicentric

20 and 30 µM of cyproterone acetate ($P<0.05$).

The results of the present investigation reveal that cyproterone acetate is potent enough to cause genotoxic damage in human lymphocytes both in the absence as well as presence of metabolic activation system (S9 mix). However there is no information available for the cause of its genotoxicity, but induction of DNA repair was reported in primary rat hepatocytes exposed to synthetic progestins (Martelli et al. 2003). Synthetic progestins like megestrol acetate and chlormadinone acetate also shows the formation of DNA adducts in primary cultures of human hepatocytes (Werner et al. 1997). Metabolism of estrone-3,4-quinone produces free radicals in human breast cancer cells (MCF-7) and these are responsible for chromosomal damage (Nutter et al. 1994). Other catechol estrogens are reported to induce DNA damage by generation of reactive oxygen species or free radicals (Thibodeau and Paquette 1999; Chen et al. 1998; Han and Liehr 1995; Li et al. 1994; Roy et al. 1991). Certain synthetic steroids have been reported to be mutagenic in the Ames tester strains by generating reactive oxygen species in the system (Islam et al. 1991). A significant increase in the

**Table 2: Structural chromosomal aberrations (CAs) Abnormal in human lymphocytes treated with cyproterone acetate in the presence of S9 mix**

| *Treatment* | *Cells scored* | *Abnormal cells (%)* | *Total structural CA* | | | | | *Total breaks without gaps (%)* |
|---|---|---|---|---|---|---|---|---|
| | | | *Gaps* | *CTB* | *CSB* | *CTE* | *DIC* | |
| *Cyproterone acetate* | | | | | | | | |
| 5 µM | 300 | 11 (3.67) | 8 | 11 | 2 | - | - | 13 (4.34) |
| 10 µM | 300 | 13 (4.34) | 12 | 19 | 4 | - | - | 23 (7.67) |
| 20 µM | 300 | 37 (12.34)* | 34 | 43 | 13 | 5 | - | 66 (22.00) |
| 30 µM | 300 | 41 (13.67)* | 39 | 49 | 21 | 6 | - | 82 (27.34) |
| Untreated | 300 | 4 (1.34) | 3 | 2 | - | - | - | 2 (0.67) |
| *Negative control* | | | | | | | | |
| DMSO (5 µl/ml) | 300 | 5 (1.67) | 4 | 3 | 1 | - | - | 4 (1.34) |
| *Positive control* | | | | | | | | |
| Cyclophosphamide ($0.5x10^{-5}$ M) | 300 | 129 (43)** | 47 | 56 | 38 | 6 | 4 | 114 (38.00) |

Significant at *$P < 0.005$, ** $P < 0.001$ Vs Untreated (chi-square test)
DMSO: Dimethylsulphoxide; CTB: Chromatid break; CSB: Chromosome break; CTE: Chromatid exchange; DIC: Dicentric

**Table 3: Frequency of sister chromatid exchanges (SCEs) in cultured human lymphocytes exposed to cyproterone acetate.**

| *Group* | *Metaphases scanned* | *SCEs/Cell (mean±SE)* | *Group* | *Metaphases scanned* | *SCEs/Cell (mean±SE)* |
|---|---|---|---|---|---|
| *Without S9 mix* | | | *With S9 mix* | | |
| *Cyproterone acetate* | | | *Cyproterone acetate* | | |
| 5 µM | 50 | 3.04±0.26 | 5 µM | 50 | 3.53±0.27 |
| 10 µM | 50 | 4.02±0.34 | 10 µM | 50 | 3.89±0.33 |
| 20 µM | 50 | 5.30±0.43* | 20 µM | 50 | 5.32±0.43* |
| 30 µM | 50 | 7.83±0.48* | 30 µM | 50 | 8.01±0.50* |
| Untreated | 50 | 2.31±0.21 | Untreated | 50 | 2.71±0.23 |
| *Negative control* | | | *Negative control* | | |
| DMSO (5 µl/ml) | 50 | 3.01±0.24 | DMSO (5 µl/ml) | 50 | 2.36±0.22 |
| *Positive control* | | | *Positive control* | | |
| Mitomycin C (0.3 µg/ml) | 50 | 10.31±0.53* | Cyclophosphamide ($0.5x10^{-5}$ M) | 50 | 12.43±0.57* |

*Significant with respect to untreated P – 0.05 (student's t test).
DMSO: dimethylsulphoxide.
*Symbols:*
µl = micro liter µM = micro molar w/v = weight per volume µg = microgram M = molar ml = milliliter

number of lymphocytes with DNA migration in the alkaline comet assay and frequency of sister chromatid exchanges per metaphases were observed in oral contraceptive users as compared with their age matched untreated controls (Biri et al. 2002). Norgestrel and other synthetic estrogens induces chromosomal aberrations and sister chromatid exchanges both in the presence as well as absence of metabolic activation system (Ahmad et al. 2001; Hundal et al. 1997; Siddique and Afzal 2004).

The results obtained in this study suggests a genotoxic potential of cyproterone acetate. It is, therefore, advisable to be careful of the potential hazards of the cyproterone acetate. This demands the lowest possible use of effective and acceptable doses of these drugs so as to minimizes any potential risk. Otherwise they may become capable of attacking the genetic material.

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