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Mutation Research 370 (1996) 175–180

MR
Genetic Toxicology

Genotoxicity of lynoral (ethinyloestradiol, an oestrogen) in mouse bone marrow cells, in vivo

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Received 4 April 1996; revised 29 May 1996; accepted 30 May 1996

Abstract

The genotoxic effect of Lynoral (ethinyloestradiol, an oestrogen) was studied using mouse bone marrow cells treated in vivo, employing a chromosomal aberration assay and micronucleus test. The dose and time-yield effects of the sex hormonal drug were investigated. Lynoral failed to induce significant genetic damage in the bone marrow erythrocytes of mice, regarding chromosomal aberrations and micronuclei.

Keywords: Lynoral; Oestrogen; Ethinyloestradiol; Micronucleus; Chromosomal aberration

1. Introduction

The mutagenic potential of sex hormonal drugs has been assayed considerably in recent years. Oral contraceptives with varying concentrations of oestrogens and progestins are reported to be mutagenic, both in plants and animals [1–4]. The genotoxic effect of oestrogens [5–7] and progestins [8,9] as individual compounds has also been reported.

The genotoxic and carcinogenic effects of oestrogen have been studied by various authors. Oestradiol-induced chromosomal aberrations have been reported by Serova and Kerkis [10] in human embryonal fibroblasts and kidney epithelial cells. 17β-Oestradiol-induced meiotic chromosomal aberrations have been reported by Rahman and Rajasekarasetty [11] in grasshoppers. Administration of oestrogens leads to an increased level of endometrial cancer in women, and oestradiol can be expected to have the same effect [5]. Oestradiol is found to induce very low frequencies of hyperploidies in human synovial cells [12].

Oestrogen was negative in the Ames test [13]. Oestradiol did not induce either chromosomal aberrations [14] or sister chromatid exchanges [15] in human lymphocytes. The oestrogens, 17β-oestradiol, oestrogen, oestradiol and DES are found to be toxic, but not mutagenic in vitro using Chinese hamster cells [16].

Thus, the reports on the genotoxic effects of oestrogens are controversial. Hence the present work was undertaken to add more information about the genotoxic potential of oestrogen, in particular that of ethinyloestradiol on the bone marrow cells of Swiss albino mice, using a chromosomal aberration assay and micronucleus test.

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PII S0165-1218(96)00065-1

2. Materials and methods

In the present investigation, in vivo studies were carried out in Swiss albino mice (*Mus musculus*), 8–10 weeks old, with an average body weight of 28 g. The animals were bred and maintained in our laboratory. These animals were housed in polypropylene cages (290 × 220 × 140 mm) bedded with paddy husk and were maintained at a temperature of 28(±2)°C and 55(±5)% humidity. The animals were fed with standard mouse pellets (Lipton India) and water ad libitum. Five animals were used for each treatment and control group.

Lynoral (INFAR, Batch No. 274178) tablets consisting of 0.05 mg ethinyloestradiol, a semisynthetic oestrogen, were used as the test chemical. These are used mainly in the treatment of prostatic cancer and other oestrogen deficiency symptoms, by oral administration. Lynoral tablets were dissolved in sterilized double distilled water to form suspensions of various concentrations viz. 0.002, 0.010, 0.020, 0.040, 0.080, 0.120, 0.120, 0.160 and 0.200 mg/kg body weight. The lowest concentration, 0.002 mg/kg, represents the human therapeutic dose. All the concentrations doses were administered to mice for 15 consecutive days using an oral catheter. Dose-response analysis was carried out for the above concentrations 24 h after the final feeding. Time-response studies were done using mice fed with 0.02 mg/kg/day, at 6, 12, 24, 48, 96 h and 1, 2 and 3 weeks after the final feeding.

Animals fed with single dose of 50 mg/kg of cyclophosphamide (CP; Endoxan, Asta-Werke AG, Germany, Batch No. 707032) were used as positive controls. Time-response studies of CP were carried out at 6, 24 and 48 h after feeding. Animals fed with sterile double-distilled water served as negative solvent controls.

2.1. Chromosome analysis

Mice were injected intraperitonially with 0.2 ml of 0.025% colchicine, 1.5 h before they were killed by cervical dislocation. Bone marrow chromosome preparation was conducted according to the method of Tjio and Whang [17], using 0.56% Potassium chloride as hypotonic solution. Flame-dried slides were coded and stained with buffered 10% Giemsa (pH 6.8). 100 metaphase spreads per animal were analysed. Various kinds of chromosomal aberrations induced by the drugs were identified and analysed, as per the details of Savage [18]. Dislocated and misaligned chromosomal fragments which are not clearly associated with any exchange processes were

Table 1
Percentage [a] of chromosomal aberrations induced by various doses of Lynoral at 24 h after treatment

Nature of treatment	Dose (mg/kg b.w.)	MI [b]	Chromosomal aberrations			
			BS	EX	MA	Total ± SD
Control	–	3.12	1.40	0.70	–	2.10 ± 0.32
Lynoral	0.002 [c]	2.80	1.36	0.68	–	2.04 ± 1.11
Lynoral	0.010	2.85	2.68	2.01	–	4.69 ± 1.30
Lynoral	0.020	2.62	2.80	2.10	–	4.90 ± 1.53
Lynoral	0.040	2.98	3.85	1.72	–	5.57 ± 1.75
Lynoral	0.080	2.78	2.80	2.80	–	5.60 ± 1.68
Lynoral	0.120	2.79	5.21	2.08	–	7.29 ± 1.90 *
Lynoral	0.160	2.76	7.45 **	3.11	–	10.56 ± 1.99 **
Lynoral	0.200	2.69	9.20 **	3.45	–	12.65 ± 2.30 ***
CP	50.00	2.12	4.67	5.61 *	1.87	12.15 ± 2.11 ***

[a] From 100 metaphases/animal.
[b] From 2000 cells/animal.
[c] Human therapeutic dose.
BS, breaks; EX, exchanges; MA, multiple aberrations; S & P, stickiness and pulverizations
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

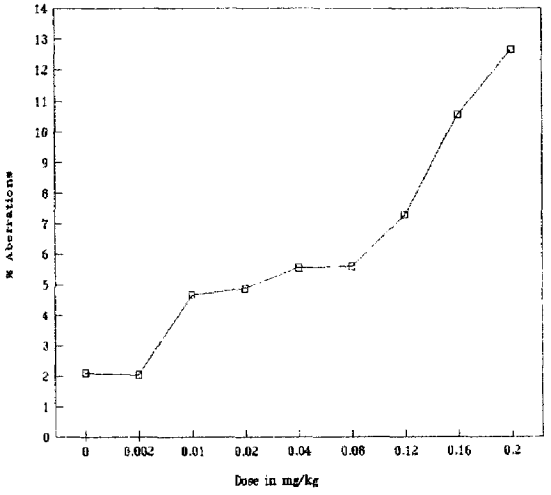

Fig. 1. Dose-response curve for chromosomal aberrations induced by Lynoral at 24-h treatment.

included under breaks. Only the interchromosomal exchanges were scored under exchanges. The mitotic index (MI) was calculated by analysing 2000 cells per animal.

2.2. Micronucleus test

Experimental animals were killed at different time intervals. Bone marrow preparations were made according to the method of Schmid [19], with a slight modification, i.e., the fetal calf serum was replaced by 5% bovine serum albumin solution in phosphate-buffered saline (PBS) [20]. Smears were stained with May-Grunwald Giemsa and analysed for the presence of MN in both polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs). 2000 PCEs and the corresponding number of NCEs per animal were analysed for the presence of MN. The ratio of PCEs and NCEs (P/N) was calculated for all treated and control groups.

2.3. Statistical analysis

Statistical analysis of the data on chromosomal aberrations was performed using the Chi-square test [21]. For the micronucleus test results, the Mann-Whitney *U*-test was employed.

3. Results

3.1. Chromosome analysis

Dose-response assay. The results of the analysis of the chromosomal aberrations induced by various doses of lynoral at 24 h after a 15-day treatment are summarized in Table 1. The dose-response curve for chromomosomal aberrations is reported in Fig. 1. Except for the very high doses (0.120 mg/kg/day and above), the drug failed to induce any statistically significant increase in classical aberrations at any of the doses ranging from the human therapeutic dose (0.002 mg/kg/day) up to 40 times this dose (0.080 mg/kg/day). The MI values do not show any sig-

Table 2
Percentage [a] of chromosomal aberrations induced by Lynoral (0.02 mg/kg) at different time intervals after treatment

Nature of treatment	Time	MI [b]	Chromosomal aberrations			
			BS	EX	MA	Total ± SD
Control	6 h	2.95	1.54	0.31	–	1.85 ± 0.79
Lynoral	6 h	2.10	2.05	2.05	–	4.10 ± 0.51
CP	6 h	2.90	3.80	2.53	–	6.33 ± 0.65
Control	12 h	3.25	1.99	0.33	–	2.32 ± 0.32
Lynoral	12 h	2.49	0.85	6.78 ***	–	7.63 ± 0.69 *
Control	24 h	3.12	1.43	0.72	–	2.15 ± 0.39
Lynoral	24 h	2.62	2.80	2.10	–	4.90 ± 0.49
CP	24 h	2.12	4.67	5.61 **	1.87	12.15 ± 1.89 ***
Control	48 h	2.89	1.72	0.34	–	2.36 ± 0.36
Lynoral	48 h	2.61	1.27	0.00	–	1.27 ± 0.24
CP	48 h	2.68	3.70	1.23	1.23	6.16 ± 0.89
Control	96 h	2.65	1.49	0.00	–	1.49 ± 0.21
Lynoral	96 h	2.46	1.55	0.52	–	2.07 ± 0.31
Control	1 w	2.90	0.99	0.00	–	0.99 ± 0.18
Lynoral	1 w	2.57	2.94	0.00	–	2.94 ± 0.29
Control	2 w	3.20	1.96	0.00	–	1.96 ± 0.15
Lynoral	2 w	2.85	2.50	1.25	–	3.75 ± 0.37
Control	3 w	3.15	1.48	0.00	–	1.48 ± 0.15
Lynoral	3 w	2.90	2.11	2.11	–	4.22 ± 0.44

[a] From 100 metaphases.
[b] From 2000 cells/animal.
Description of aberrations as in Table 1.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3
Percentage [a] of MN induced by Lynoral at different doses at 24 h after treatment

Nature of treatment	Dose (mg/kg b.w.)	Mean %PCE	Mean %NCE	Mean % MN in PCE ± SD	Mean % MN in NCE ± SD	Mean P/N ± SD
Control	–	50.68	49.32	0.29 ± 0.038	0.15 ± 0.032	1.03 ± 0.043
Lynoral	0.002 [b]	50.32	49.68	0.27 ± 0.042	0.13 ± 0.009	1.02 ± 0.070
Lynoral	0.010	55.16	44.84	0.36 ± 0.055	0.19 ± 0.000	1.23 ± 0.026
Lynoral	0.020	55.56	44.44	0.30 ± 0.032	0.24 ± 0.009	1.26 ± 0.083
Lynoral	0.040	54.37	45.63	0.26 ± 0.095	0.19 ± 0.000	1.19 ± 0.036
Lynoral	0.080	50.56	49.44	0.24 ± 0.052	0.16 ± 0.006	1.05 ± 0.013
Lynoral	0.120	51.35	48.65	0.20 ± 0.038	0.19 ± 0.021	1.06 ± 0.028
Lynoral	0.160	52.65	47.35	0.21 ± 0.048	0.22 ± 0.044	1.12 ± 0.084
Lynoral	0.200	51.80	48.20	0.20 ± 0.053	0.20 ± 0.038	1.07 ± 0.063
CP	50.0	38.75	61.25	3.63 ± 0.150 *	1.43 ± 0.093 *	0.63 ± 0.012

[a] From 2000 PCE/animal.
[b] Human therapeutic dose.
* $p = 0.008$.

Table 4
Percentage [a] of MN induced by Lynoral (0.020 mg/kg) at different time intervals after treatment

Nature of treatment	Time intervals	Mean % PCE	Mean % NCE	Mean % MN in PCE ± SD	Mean % MN in NCE ± SD	Mean P/N ± SD
Control	6 h	50.82	49.18	0.27 ± 0.046	0.13 ± 0.038	1.03 ± 0.058
Lynoral	6 h	51.10	48.90	0.21 ± 0.052	0.10 ± 0.018	1.06 ± 0.074
CP	6 h	44.31	55.69	0.74 ± 0.086	0.34 ± 0.030	1.60 ± 0.096
Control	12 h	51.23	48.77	0.28 ± 0.034	0.14 ± 0.036	1.05 ± 0.043
Lynoral	12 h	52.39	47.61	0.24 ± 0.017	0.12 ± 0.032	1.11 ± 0.091
Control	24 h	50.68	49.32	0.29 ± 0.038	0.15 ± 0.032	1.03 ± 0.043
Lynoral	24 h	55.56	44.44	0.30 ± 0.012	0.19 ± 0.009	1.26 ± 0.083
CP	24 h	38.75	61.25	3.63 ± 0.150 *	1.43 ± 0.093 *	0.63 ± 0.012
Control	48 h	51.84	48.16	0.31 ± 0.042	0.17 ± 0.013	1.06 ± 0.034
Lynoral	48 h	47.83	52.19	0.24 ± 0.038	0.19 ± 0.006	1.08 ± 0.023
CP	48 h	36.34	63.66	3.17 ± 0.126 *	1.28 ± 0.113 *	0.57 ± 0.011
Control	96 h	51.65	48.35	0.27 ± 0.000	0.14 ± 0.034	1.07 ± 0.056
Lynoral	96 h	48.21	51.79	0.21 ± 0.038	0.10 ± 0.058	0.94 ± 0.081
Control	1 w	50.15	49.85	0.26 ± 0.026	0.13 ± 0.043	1.01 ± 0.043
Lynoral	1 w	43.16	56.84	0.18 ± 0.035	0.11 ± 0.013	0.76 ± 0.022
Control	2 w	49.89	50.11	0.28 ± 0.034	0.15 ± 0.041	1.00 ± 0.034
Lynoral	2 w	49.56	50.44	0.16 ± 0.032	0.10 ± 0.006	0.89 ± 0.050
Control	3 w	51.03	48.97	0.30 ± 0.043	0.18 ± 0.047	1.04 ± 0.023
Lynoral	3 w	46.44	53.36	0.15 ± 0.003	0.09 ± 0.006	0.88 ± 0.069

[a] From 2000 PCE/animal.
* $p = 0.008$.

nificant changes. The positive control, CP (Endoxan), induced significant number of chromosomal aberrations as well as a considerable reduction in the mitotic index.

Time-response assay. Table 2 summarizes the results of the chromosomal aberrations induced by lynoral (0.02 mg/kg) at different time intervals. Except at 12 h after treatment, there were no significant frequencies of aberrations were observed. The MI values did not show any significant changes at any of the time-intervals studied. CP induced significant aberrations at 24 and 48 h. No significant difference in the MI values was noted, except at 24 h, for CP-treated mice.

3.2. Micronucleus test

Dose-response assay. Table 3 indicates the frequency of MN induced by various doses of lynoral at 24 h after 15 days of treatment. Lynoral did not induce any statistically significant increase in the number of MN compared to negative controls. No significant difference in P/N ratio was observed. CP induced a significant frequency of MN.

Time-response assay. The frequencies of MN induced by lynoral (0.02 mg/kg) at different time intervals can be seen in Table 4. No significant increase in micronucleated erythrocytes was noted at any of the time intervals studied. The P/N ratio did not show any significant change. CP induced a significant frequency of MN.

4. Discussion

According to the present investigation, lynoral failed to induce a significant frequency of chromosomal aberrations in the bone marrow cells of mice either at the human therapeutic dose or at all the higher doses upto 40 times of this human therapeutic dose. In addition, the drug did not induce any significant frequency of micronuclei, at any of the doses studied. The chromosomal aberrations and micronuclei induced by CP in significant quantities indicate the sensitivity of the strain of mouse and the parameters used for the present study.

The dose-response curve (Fig. 1) indicates that at high doses, i.e., 0.120 mg/kg and higher of Lynoral induced a linear increase in chromosomal aberrations.

This inability of Lynoral to induce genotoxicity in the present study is in agreement with the following earlier reports from related studies. Ingerowski et al. [13] showed that oestrogens were not mutagenic to bacteria, using the Ames test. Oestradiol did not induce sister-chromatid exchanges in mammalian cells, including human peripheral lymphocytes [15]. Banduhn and Obe [14] reported that no structural chromosomal aberrations were induced by oestradiol and diethylstilbestrol (DES) in human leukocyte cultures either in the presence or absence of metabolic activation. These observations in human leukocytes are very much in line with our present observations, although we studied it using mouse erythrocytes. In line with the present results, Drevon et al. [16] found oestrogens such as 17β-oestradiol, oestrogen, oestradiol and DES to be non-mutagenic in vitro, although they were toxic to Chinese hamster cells.

The present data for the micronucleus test clearly indicate that lynoral failed to induce significant frequencies of MN at any of the dose and time intervals studied. This supplements the non-mutagenicity of the drug in the present study using a chromosomal aberration test. It may be that the deletions induced by high doses, i.e., 0.160 and 0.200 mg/kg of lynoral at 24 h might have given rise to an increase in MN at a later period of time, after the immediate next cell division.

Thus it may be concluded that lynoral is not mutagenic at human therapeutic doses or upto 40 times this, although at exceptionally high doses it may induce genotoxicity, directly or indirectly.

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