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In vivo induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) by lynestrenol

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Genotoxicity study of synthetic progestin lynestrenol, was carried out on mouse bone marrow cells using sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) as parameters. Lynestrenol was studied at three different doses (6.87, 13.75 and 27.50 mg/kg body wt.). SCE and CA increased significantly as compared to normal control when treated with lynestrenol at 13.75 and 27.50 mg/kg body wt. The present results suggest that lynestrenol has both a genotoxic and cytotoxic effects in mouse bone marrow cells.

Keywords: Chromosomal aberrations, Genotoxicity, Lynestrenol, Mouse bone marrow cells, Sister chromatid exchanges

Synthetic progestins are widely used as oral contraceptives in addition to their use in the treatment of various menstrual disorders, various types of cancers and in hormonal replacement therapy. For contraception, these are either used alone or in combination with estrogens. Progestins, like estrogens diffuses easily across the cell membranes and bind to highly specific, soluble receptor proteins in the cytoplasm. The steroid receptor complex modifies the expression of specific genes by binding to control elements in DNA[1]. Lynestrenol is used either as single entity drug or in combination with estrogen, such as ethinyl estradiol or mestranol in oral contraceptives[2]. Lynestrenol was reported to protect pure bred female beagle dogs against the development of mammary tumors. At intermediate and high doses, lynestrenol was associated with increased incidence of mammary nodules and carcinomas[3]. A significant increase in the incidence of hepatocellular adenomas has been reported in male mice who were treated orally with lynestrenol for 20 months[4]. It induces chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) in human lymphocytes in the presence of metabolic activation system[5]. In the present study, an attempt has been made to study the effect of lynestrenol on sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) frequencies in mouse bone marrow cells.

Chemical — Lynestrenol (CAS No.: 52-76-6; Wyeth Lab); dimethyl sulphoxide (DMSO, 0.1 ml/animal, E. Merck, India); colchicine (6.0 mg/kg body wt., Microlab); Hoechst 33258 stain (0.05 w/v, Sigma); 3 and 7% of giemsa solution in phosphate buffer (*p*H 6.8, E. Merck, India); N-methyl-N-nitro-N′-nitrosoguanidine (MNNG, 1.2×10^4 µg/kg body wt., Sigma); 5-bromo-2-deoxyuridine (BrdU, 1.6 g/kg body wt., Sigma) were used.

Animals —Swiss albino female mice (*Mus musculus* L.) weighing 25-30 g, 10-12 weeks old were procured from Iradat Nagar (Daliganj) Lucknow, India and grouped in different cages (5 animals/group; polypropylene cages) and kept at 25°C.

Sister chromatid exchanges (SCEs) analysis — The fluorescent plus Giemsa techniques[6] were followed with slight modification for SCE analysis. 5-Bromo-2-deoxy-uridine in tablet form (BrdU; 1.6 g/kg body weight) was implanted (sc) in the neck region of each mouse under mild anaesthesia and after 30 min, lynestrenol at 6.87, 13.75 and 27.50 mg/kg body wt was injected intraperitoneally (ip), to different groups of animals. According to the LD_{50} dose (110 mg/kg body wt) was obtained with the method of Lorke[7], and highest tested dose used in the present study corresponded to 1/4 of LD_{50} dose. DMSO (0. 1 ml) as negative control and MNNG (1.2×10^4 µg/kg body wt.) as positive control were injected intraperitoneal (ip) to different groups of mice. After 21 hr, the animals received an injection (ip) of colchicine (6.0 mg/kg body wt.) and after 3 hr, the bone marrow of femur from treated animals were obtained in KCl (0.075 *M*) at 37°C and kept for 30 min at the same temperature. Material was centrifuged at 1000 rpm at room temperature and the supernatant was removed. Fixative (methanol:glacial acetic acid; 3:1; 5 ml) was added. The fixative was removed by centrifugation and 1000 rpm at room temperature and the procedure was repeated twice. Slides were stained for 20 min in 0.05% (w/v) Hoechst 33258 solution, rinsed with tap water and placed under a UV lamp, for 90 min, cov-

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ered with 'Sorensen's buffer (*p*H 6.8) and stained with 3% of giemsa solution in phosphate buffer (*p*H 6.8) for 15 min. SCE average was taken from an analysis of metaphase during second cycle of divisions. At least for 60 sec mitosis division per mouse were scored to determine the frequency of SCEs.

Chromosomal aberrations (CAs) analysis —For analysis of chromosomal aberrations, tested and control doses were the same as described for SCE analysis. All animals were injected (ip) with colchicine (6.0 mg/kg body wt) and sacrificed by cervical dislocation after 2 hr before sacrifice. Bone marrow preparations for the analysis of chromosomal aberrations in metaphase cells were obtained by the technique of Yosida and Amano[8]. Slides were stained with 7% of giemsa stain in phosphate buffer (*p*H 6.8). Five animals were taken for each treatment and 100 well spread metaphases containing (40±2) chromosomes were analysed per animal.

Statistical analysis —The significance of the difference between experimental and control data was calculated using the t test. The level of significance was tested from standard statistical table of Fisher and Yates[9].

Lynestrenol at 13.75 and 27.50 mg/kg body wt. induced significant sister chromatid exchanges (SCEs) and chromosomal aberrations (CAs) in the treated group as compared to untreated group (Tables 1, 2). At 6.87 mg/kg body wt the values of SCEs/cell and CAs were not significantly higher than control (Tables 1, 2). MNNG and DMSO induced 11.86±1.51 and 2.24±0.12 SCEs per cell, respectively.

Similar observations on DNA damaging properties of steroids as evident from chromosomal damage, induction of SCEs[10-13] and formation of endogenous DNA adducts and certain neoplastic changes have also been reported earlier[14]. Some drugs are unable to induce significantly higher frequencies of chromosomal aberrations and micronuclei formation in bone marrow cells of rats[15]. Estrone 3,4-quinone produces free radicals in human breast cancer cells (MCF-7) and these are responsible for chromosomal DNA damage[16]. Other catechol estrogen are reported to induce DNA damage by generating reactive oxygen

Table 1— Frequency of sister chromatid exchanges (SCEs) induced by lynestrenol in mice bone marrow.

Treatment	No. of metaphases analyzed	SCEs/ metaphase $^{\Psi}$	Range
Lynestrenol (mg/kg body weight)			
6.87	300	3.78 ± 0.29	1 – 7
13.75	300	7.21 ± 0.73*	2 – 7
27.50	300	8.68 ± 0.97*	3 – 9
Untreated	300	2.01 ± 0.11	1 – 5
Positive control MNNG (1.2×10^4 µg/kg body weight)	300	11.86 ±1.51*	3 – 10
Negative control (DMSO 0.1 ml/animal)	300	2.24 ± 0.12	1 – 5
BrdU (1.6 g/kg body weight)	300	2.73 ± 0.15	1 – 5

Significant at *$P<0.001$ vs untreated.
MNNG: N-methyl-N-Nitro-N′-nitrosoguanidine; DMSO: dimethylsulphoxide
BrdU: 5-bromo-2-deoxyuridine; $^{\Psi}$: Values are mean ± S E

Table 2 — Mean percentage of chromosomal aberrations in mouse bone marrow cells after lynestrenol treatment

Treatment	Abnormal metaphases without gaps		Chromosomal aberrations			
	Number	Percentage $^{\Psi}$	Gaps		Fragments and or breaks	
			Number	%	Number	%
Lynestrenol (mg/kg body weight)						
6.87	10	2 ± 0.62	6	1.2	10	2
13.75	53	10.6 ± 1.37*	19	3.8	35	7
27.50	79	15.8 ± 1.63*	23	4.6	51	10.2
Untreated	7	1.4 ± 0.52	5	1	7	1.4
Positive control MNNG (1.2×10^4 µg/kg body weight)	141	28.2 ± 2.01*	37	7.4	89	17.8
Negative control (DMSO 0.1 ml/animal)	11	2.2 ± 0.65	5	1	6	1.2

One hundred cells were analyzed per animal for a total of 500 cells per treatment.
*Significant difference from the untreated ($P<0.01$).
MNNG: N-methyl-N-Nitro-N′-nitrosoguanidine; DMSO: dimethylsulphoxide
$^{\Psi}$: Values are mean ± S E

species by free radicals[17-21]. Synthetic progestins like megestrol acetate and chlormadinone acetate also shows the formation of DNA adducts in the primary cultures of human hepatocytes[22]. A significant increase in the number of lymphocytes with DNA migration in alkaline comet assay and frequency of sister chromatid exchanges (SCEs) per metaphase have been observed in the oral contraceptives user as compared with their age matched untreated controls[23]. Most of the chromosomal aberrations observed in cells are lethal, but there are many corresponding aberrations that are viable and can cause genetic effects, either somatic or inherited[24]. In earlier study, lynestrenol has been reported to be genotoxic in the presence of metabolic activation system (S9 mix) in human lymphocytes *in vitro*[5], so possibly the metabolic activation of it converts lynestrenol into possible reactive species that interact with DNA and cause the genotoxic damage, as was evident in the mice bone marrow cells in the present study.

Hence, it is suggested to use minimum effective and acceptable doses of these drugs so as to minimize any potential risk.

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References

1 Yamamoto K R, Steroid receptor regulated transcription of specific genes and gene networks. *Annu Rev Genet*, 19 (1985) 209.

2 IARC, *IARC monograph on hormonal contraception and post menopausal hormonal therapy*, vol. 72 (IARC, Lyon, France) 1999, 49.

3 Misdorp W, Progestogens and mammary tumours in dogs and cats, *Acta Endocrinol*, 125 (1991) 27.

4 Schuppler J & Gunzel P, Liver tumors and steroid hormones in rats and mice, *Arch Toxicol*, 2 (1979) 181.

5 Siddique Y H & Afzal M, Evaluation of genotoxic potential of synthetic progestin lynestrenol in human lymphocytes *in vitro*, *Indian Biol*, 36, (2004)11

6 Perry P & Wolff P, New Giemsa method for the differential staining of sister chromatids, *Nature*, 261 (1974) 156.

7 Lorke D, A new approach to practical acute toxicity testing, *Arch Toxicol*, 54 (1983) 275.

8 Yosida T H & Amano K, Autosomal polymorphism in laboratory bred and wild. Norway rats, *Rattus norvegicus* Misima, *Chromosoma*, 16 (1965) 658.

9 Fisher R A & Yates F, *Statistical table for biological, agricultural and medical research*, 6th edition (Oliver and Boyd, Edinburg) 1963, 138.

10 Singh H, Singh J R, Dhillon V S, Bali D & Sharma H P, *In vitro* and *in vivo* genotoxicity evaluation of hormonal drug II. Dexamethasone, *Mutat Res*, 308 (1994) 89.

11 Dhillon V S, Singh J R, Singh H & Kler R S, *In vitro* and *in vivo* genotoxicity evaluation of hormonal drugs V, mestranol, *Mutat Res*, 322 (1994) 173.

12 Lehmann M, Putz B, Poggel M A & Gunzel P, Experimental toxicity studies with contraceptives of steroids and their relevances for human risk estimation, in *Advances in applied toxicology*, edited by A D Dayam and A J Paine (Taylor and Frances, London) 1989, 51.

13 Ahmad M E, Shadab G G H A, Azfer M A & Afzal M, Evaluation of genotoxic potential of synthetic progestins-norethindrone and norgestrel in human lymphocytes *in vitro*. *Mutat Res*, 494 (2001) 13.

14 Martelli A, Mattioli F, Angiola M, Reimann R & Brambilla G, Species, sex and inter-individual differences in DNA repair induced by nine sex steroids in primary cultures of rats and human hepatocytes, *Mutat Res* 536 (2003) 69.

15 Herrera J A, Coco R & Bergada C, Evaluation dela possible mutagenicidal del acetate de medroxi progesterone por la prueda de micronucleus, *Medicina*, 40 (1980) 774.

16 Nutter L M, Wu Y Y, Ngo E O, Sierra E E, Gutiezrrez P L & Abul Hajj Y, A o-quinone form of estrogen produces free radicals in human breast cancer cell: Correlation with DNA damage, *Chem Res Toxicol*, 7 (1994) 23.

17 Thibodeau P A & Paquette B, DNA damage induced by catechol estrogens in the presence of copper(II): Generation of reactive oxygen species and enhancement by NADPH, *Free Radic Biol Med*, 27 (1999) 1367.

18 Chen Y, Shen L, Zhang F, Lau S S, Van Breemen R B, Nikolic D & Bolton J L, The equine estrogen metabolite 4-hydro-equilenin causes DNA single strand breaks and oxidation of DNA bases, *Chem Res Toxicol*, 11 (1998) 1105.

19 Han X & Liehr J G, Microsome mediated 8-hydroxylation of guanine bases of DNA by steroid estrogens: Correlation of DNA damage by free radicals by metabolic activation to quinones, *Carcinogenesis*, 16 (1995) 2571.

20 Li Y, Trush M A & Yager J D, DNA damage caused by reactive oxygen species originating from a copper dependent oxidation of the 2-hydroxy catechol of estradiol, *Carcinogenesis*, 15 (1994) 1421.

21 Roy D, Floyd R A & Liehr J G, Elevated 8-hydroxy deoxyguanosine levels in DNA of diethylstillbestrol treated Syrian hamsters: Covalent DNA damage by free radicals generated by redox cycling of diethyl stilbestrol, *Cancer Res*, 51 (1991) 3882.

22 Werner S, Kunz S, Beckurts T, Heidecke C D, Wolff J & Schwarz L R, Formation of DNA adducts by cyproterone acetate and some structural analogues in primary cultures of human hepatocytes, *Mutat Res*, 395 (1997) 179.

23 Biri A, Civelek E, Karahalil B & Sardas S, Assessment of DNA damage in women using oral contraceptives, *Mutat Res*, 521 (2002) 113.

24 Swierenga S H H, Heddle J A, Sigal E A, Gilmann J P W, Brillinger R L, Douglas G R & Nestmann E R, Recommended protocols based on a survey of current practice in genotoxicity testing laboratories IV, chromosome aberration and sister chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocytes cultures, *Mutat Res*, 246 (1991) 301.