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CYTOGENETIC CHANGES IN BONE MARROW AND FIBROBLAST CELLS OF BALB / C LABORATORY MICE INDUCED BY MESTRANOL

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In this work the genotoxic effects provoked by Mestranol, which belongs to the group of chemical rodenticide- chemosterilants, were examined as a function of dose (7,5, 15 and 30 mg/kg w.b./c.c.) and of time (10, 20 and 30 days or 3, 5 and 7 days for "in vivo" and "in vitro" conditions, respectively). The genotoxic effects were scored on the basis of numerical and structural aberrations in both bone marrow and fibroblast cells from BALB/C laboratory mice in comparison to control groups.

Increased doses of the preparation increased numerical aberrations in both bone marrow and fibroblast cells significantly ($p<0.01$) so that the greatest changes were noticed after the treatment with 30 mg/kg w.b./c.c. Numerical aberrations showed no significant differences ($p<0.05$) as a function of time. Also, the several times greater number of aneuploid cellss was significantly different ($p<0.01$) compared to the number of polyploid cells

Greater values for structural aberrations were obtained not only after the treatment with the largest dose of 30 mg/kg w.b./c.c., but also after longer exposure times (i.e. for 30 or 5 days respectively). Statistically significant differences were found between the number of gaps, as well as fragments, in comparasion to the number of Robertsonian translocations ($p<0.01$), while there were no significant differences between thenumber of gaps and fragments ($p>0.05$).

Key words: cytogenetic changes, Mestranol, mouse, fibroblast, bone marrow cells.

INTRODUCTION

After several decades of application the use of earlier mechanical, physical, biological and chemical methods to eradicate rodents, no longer gives satisfactory results, especially of the application chemical preparations, because resistance occurrs of more and more frequently. For that reason a new group of chemical preparations was developed on the basis of chemosterilants, which induce temporary or permanent sterility (Eriksson *et al*., 1971). Chemosterilants can function at various stages of the reproductive cycle: at the gamete level, before or after fertilization, by preventing gamete implantation into the uterus by

damaging the embryo during development orby inducing abortion (Jakson, 1959; Rex, 1970).

During the sixties Mestranol was introduced as a chemosterilant, as well as a standard for estrogenic steroids, and during the following decade many uses of this preparation were investigated in practice. It was found that a bait treated with Mestranol was very acceptable and that the most significant reduction of young animals, occurr in 82 days after a treatment of both sexes (Kincl and Dorfman, 1965; Marsh and Howard, 1969). In contrast to Mestranol, there are many chemosterilants which are unacceptable for rodents (Gao and Short , 1993).

Various chemical compounds, including rodenticides, can cause genotoxical changes- mutagenetic, cancerogenic and teratogenic (Irving, 1973; Soldatović, 1994; Stanimirović *et al*., 1996). Some anticoagulants like Antikolin, Rosol, Rakumin-57, can cause genotoxic changes both in harmful rodents and in other mammals (Soldatović *et al*., 1981, Kataranovski, 1986). Taking into consideration that there are very few data on the genotoxic effects of chemosterilants, the aim of this work was to determine the possible cytogenetic changes in cells of mice treated with Mestranol as a representative estrogenic preparation.

MATERIAL AND METHODS

Chemosterilant

The genotoxic effects of Mestranol (17 α-ethinylestradiol-3-methilether) a highly efficient steroid chemosterilant, were investigated in vivo and vitro.

Experimental design

For the studies in vivo, the preparation was given orally by gastric tube to BALB/C mice in doses of 7,5, 15 and 30 mg/kg body mass. After 10, 20 and 30 days, bone marrow cells were examined for mitotic activity as well as numerical and structural aberrations. Chromosomal analyses were performed on cells fixed in Carnam solution and dissolved in 0,56% KCl by the method described by Hsu and Patton (1969).

For the studies in vitro, mouse fibroblasts were treated with 7,5, 15 and 30 mg Mestranol per kg cell culture for 2, 3 and 5 days. Mitotic activity and the appearance of structural and numerical aberrations were investigated. Muscle tissue from neonatal BALB/C mice aged 2-3 days was taken and fibroblast cultures prepared by the method of Durtrillah and Couturier (1989) for determination of the karyiogram.

The cell preparations were stained for 5 min in Giemsa. Groups of seven sexually mature male animals were formed for each dose and exposure time, as well as for the control groups.

Statistical analysis

The results obtained were analyzed Student's test (The Statgraphics 5.0-Statistical Graphics Corporation, USA programme).

RESULTS AND DISCUSSION

From the results obtained (Table 1) concerning to the appearance of numerical aberrations in mouse bone marrow cells "in vivo" it is obvious that Mestranol induced numerical aberrations. The relative number of diploid cells in all the control groups was almost equally high (98,33-100,00%), while single aneuploid cells were observed (1,66%), in some control groups. In no animal from the control groups were polyploid cells recorded. Thus the maximal level of numerical aberrations in the control groups was 1.66%, which can be tolerated, because a few may appear as a consequence of the methodology used.

With increased doses of the preparation, numerical aberrations in the bone marrow cells also increased, so that the greatest levels were noticed after treatment with 30 mg/kg w.b. This increased as a function of dose was statistically significant ($p<0.01$). However, although polyploid cell number as a function in dose increased compared to the control groups ($p<0.01$), the doses had no significant effect ($p>0.05$) as a function of time, while, with regard to the number of aneuploid cells it was significantly lower ($p<0.01$). Statistical analysis of the numerical aberrations as a function of time, showed no statistical significance ($p>0.05$).

The numerical aberations, after Mestranol application "in vitro", in mice fibroblast cells are presented in Table 2. The diploid number of chromosomes in the control groups was 98,15-100,00%, aneuploid ones maximally 1,85%, while polyploid cells were not registered. Along with the increase of Mestranol dose the numerical aberrations also increased, so in all cases statistically significant differences from the values for the control groups ($p<0.01$) were registered. Statistically significant differences ($p<0.01$) were also found concerning the number of aneuploid cells in comparison to polyploid cells after all the applied doses. Significantly more ($p<0.01$) aneuploid cells were recorded the doses of 15 and 30 mg/kg c.c. than with the smallest dose of 7.5 mg/kg c.c., but between the two larger doses there were no statistically significant differences ($p>0.05$). Concerning polyploidy in the fibroblast cells, statistically significant differences ($p<0.01$) were detected only between the maximal dose (30 mg/kg c.c), and lower doses (7,5 and 15 mg/kg c.c.) of chemosterilant.

The analyses of structural aberrations in mouse bone marrow and fibroblast cells as functions of time and dose are presented in Table 3. About 100 metaphase figures were analysed in each group. In the control groups, just one gap, was found and there were no structural aberrations. Statistically significant differences in comparison to the control groups ($p<0.01$) were evident for the groups treated with Mestranol. Structural aberrations were most numerous after the 30 mg/kg w.b./c.c. dose and after the longest exposures. Statistically significant differences ($p<0.01$) were recorded between the 30 mg/kg w.b./c.c. and 7.5 mg/kg w.b./c.c. doses. Also statistically significant differences between the levels of gaps and fragments in comparison to Robertsonian translocations ($p<0.01$), while the numbers of gaps and fragments were not significantly different ($p>0.05$).

Table 1. Cytogenetical effects (numerical changes) of Mestranol on bone marrow cells of the mouse

Number animals	Investigated cells	Number of chromosomes						Aneuploidy		Polyploidy	
		<40	%	>40	%	40	%	x	%	x	%
Investigated dose 7,5 mg/kg w.b.											
K	60.00	0.00	0.00	60.00	100.0	0.00	0.00	0.00	0.00	0.00	0.00
Ex 7	39.43	2.00	5.07	36.86	93.47	0.57	1.45	2.57	6.52	0.57	1.45
K	60.00	1.00	1.66	59.00	98.33	0.00	0.00	1.00	1.66	0.00	0.00
Ex 7	49.14	2.71	5.52	45.14	91.86	1.28	2.62	4.00	8.14	1.28	2.62
K	60.00	1.00	1.66	59.00	98.33	0.00	0.00	1.00	1.66	0.00	0.00
Ex 6	48.00	3.67	7.64	42.17	87.85	2.17	4.51	5.83	12.15	1.83	3.82
Investigated dose 15 mg/kg w.b.											
K	50.00	0.00	0.00	50.00	100.0	0.00	0.00	0.00	0.00	0.00	0.00
Ex 7	49.57	3.43	6.91	44.43	89.63	1.71	3.46	5.29	10.56	1.14	2.30
K	60.00	0.00	0.00	60.00	100.0	0.00	0.00	0.00	0.00	0.00	0.00
Ex 7	50.28	5.28	10.51	42.14	83.82	2.86	5.68	8.14	16.19	1.71	3.40
K	50.00	0.00	0.00	50.00	100.0	0.00	0.00	0.00	0.00	1.00	2.00
Ex 6	45.67	4.16	9.12	39.00	85.39	2.33	5.11	6.50	14.23	2.17	4.74
Investigated dose 30 mg/kg w.b.											
K	60.00	1.00	1.66	59.00	98.33	0.00	0.00	1.00	1.66	0.00	0.00
Ex 7	39.14	4.26	10.25	33.14	84.68	2.28	5.84	6.57	16.79	1.00	2.55
K	60.00	1.00	1.66	59.00	98.33	0.00	0.00	1.00	1.66	0.00	0.00
Ex 7	38.86	4.86	12.50	31.71	81.61	2.28	5.88	7.14	18.38	1.43	3.68
K	60.00	1.00	1.66	58.00	96.66	1.00	1.66	2.00	3.33	0.00	0.00
Ex 6	37.50	6.17	16.44	27.83	74.22	3.50	9.33	9.67	25.78	1.67	4.45

Treatment period 10 days

Treatment period 20 days

Treatment period 30 days

K - Control group Ex - Experimental group

Table 2. Cytogenetical effects (numerical changes) of Mestranol on fibroblast cells of the mouse

Group	Investigated cells	Number of chromosomes						Aneuploidy		Polyploidy	
		<40	%	40	%	> 40	%	x	%	x	%
Treatment period 2 days											
K	34	0	0.00	34	100.0	0	0.00	0	0.00	0	0.00
I	76	4	5.26	69	90.79	3	3.95	7	9.21	1	1.32
II	100	25	25.00	65	65.00	10	10.00	35	35.00	1	1.00
III	100	31	31.00	51	51.00	18	18.00	49	49.00	2	2.00
Treatment period 3 days											
K	50	0	0.00	50	100.0	0	0.00	0	0.00	0	0.00
I	82	20	24.39	58	70.73	4	4.88	24	29.27	2	2.44
II	81	24	29.63	51	62.86	6	7.41	30	37.04	2	2.47
III	67	23	34.33	33	49.25	11	16.42	34	50.75	3	4.48
Treatment period 5 days											
K	54	0	0.00	53	98.15	1	1.85	1	1.85	0	0.00
I	100	15	15.00	79	79.00	6	6.00	21	21.00	0	0.00
II	96	19	21.11	72	75.00	7	7.29	26	27.00	0	0.00
III	82	20	24.19	58	70.73	6	7.31	26	31.70	4	4.67

Investigated doses:
K – Control group
I – 7,5 mg/kg c.c.
II – 15 mg/kg c.c.
III – 30 mg/kg c.c.

Table 3. Cytogenetical effects (structural changes) of Mestranol on bone marrow and fibroblast cells of the mouse

Group	Structural changes (%)		
	Gaps	Fragments	Robertson's translocations
Bone marrow cells			
treatment period 10 days			
K	0.00	0.00	0.00
I	2.04	3.52	1.06
II	4.06	6.50	2.02
III	6.53	7.00	2.54
treatment period 20 days			
K	0.00	0.00	0.00
I	6.54	8.50	3.06
II	10.53	9.54	3.48
III	11.98	9.47	6.50
treatment period 30 days			
K	0.00	0.00	0.00
I	7.48	1.97	3.52
II	8.96	12.51	4.47
III	16.54	17.00	7.04
Fibroblast cells			
treatment period 2 days			
K	0.00	0.00	0.00
I	3.01	5.48	1.04
II	6.47	7.02	3.04
III	8.02	7.47	4.48
treatment period 3 days			
K	0,00	0.00	0.00
I	3.46	4.51	3.54
II	7.49	8.50	6.53
III	8.02	10.00	7.98
treatment period 5 days			
K	1.00	0.00	0.00
I	6.47	8.00	5.52
II	10.52	11.06	7.03
III	16.53	17.04	10.54

Investigated doses: I – 7,5 mg/kg w.b./c.c.
II – 15 mg/kg w.b./c.c.
III – 30 mg/kg w.b./c.c

On the basis of these results, it is evident that Mestranol, applied in the examined doses for the investigated times, can alter genetic material. Cytogenetic changes were recorded for other rodenticides as well (chemosterilants and anticoagulant rodenticides) by many authors (Sofradžija *et al*, 1989; Kataranovski, 1994; Stanimirović *et al*, 1997; Teodorović *et al*, 1999). As a consequence of these genotoxic effects, as determined here for Mestranol, pregnancy disorders are possible resulting in abortion or the production of off spring with congenital malformations. This has already been found by several authors for a number of preparations (Elias *et a*l., 1978; Brent, 1984; Hrgović *et al.*, 1991).

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CITOGENETIČKE PROMENE ĆELIJA KOŠTANE SRŽI I FIBROBLASTA KOD LABORATORIJSKIH MIŠEVA SOJA BALB/C INDUKOVANE MESTRANOLOM

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SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja genotoksičnih efekata preparata "Mestranola", koji spada u grupu hemijskih rodenticida-hemosterilanata. Ispitivanja su izvršena u funkciji doze (7,5, 15 i 30 mg/kg w.b./c.c) i vremena (10, 20 i 30 dana, odnosno 2, 3 i 5 dana) u uslovima *in vivo* i *in vitro*. Procena genotoksičnih efekata je vršena na osnovu numeričkih i strukturnih aberacija na ćelijama koštane srži i fibroblasta laboratorijskog miša soja BALB/C, u odnosu na kontrolne grupe.

Sa povećanjem doze preparata vrednost numeričkih aberacija, u ćelijama koštane srži i fibroblasta, je značajno ($p<0,01$) rasla tako da su maksimalni nivoi zabeleženi posle tretmana u dozi od 30 mg/kg w.b., dok kod numeričkih aberacija u funkciji vremena nije utvrđena statistička značajnost ($p>0,05$). Takođe su utvrđene statistički značajne razlike ($p<0,01$) u pogledu višestruko većeg broja aneuploidnih ćelija u odnosu na poliploidne.

Maksimalne vrednosti strukturnih aberacija postignute su ne samo posle tretmana sa maksimalnom dozom od 30 mg/kg w.b./c.c., već i posle najdužih ekspozicija (30, odnosno 5 dana). Utvrđene su statistički značajne razlike između nivoa nastalih gapova, odnosno fragmenata u odnosu na Robertsonove translokacije ($p<0,01$), dok u pogledu razlika nivoa nastalih gapova i fragmenata nisu utvrđene signifikantne razlike ($p>0,05$).