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THE GENOTOXIC EFECTS OF ALPHACHLORHYDRIN IN BALB/C LABORATORY MICE

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The genotoxic properties of different doses (7,5, 15 and 30 mg/kg body mass) of alphachlorchydrin were investigated in vivo in BALB/C mice and in vitro using cell cultures. Thus, mouse bone marrow cells were examined after exposure for 10/20 and 30 days under in vivo conditions, while cultures of mouse fibroblasts were examined after exposure for 2, 3 and 5 days under in vitro conditions.

The genotoxic effects of this chemosterilant vere evaluated on the basis of numerical and structural aberrations in the three experimental groups for each dose and each length of exposure in comparison with control groups. From the results obtained, it may be concluded that alphachlorchydrin in the tested doses caused genotoxic effects both in bone marrow cells and in cell cultures of mouse fibroblasts. The number of numerical aberrations increased with increase indoseor length of exposure, although the differences were not statistically significant ($p>0.05$). The increase in the number of structural aberrations in bone marrow cells from mice treated with 30 mg alphachlorhydrin per kg body mass was statistically significant ($p<0.01$) when compared with the effect of the 7.5 mg/kg dose. Significant differences concerning numerical and structural aberrations were also found between the treated groups and the control group.

Key words: genotoxicity, alphachlorchydrin, chemosterilant, mouse fibroblast, bone marrow cells.

INTRODUCTION

In these days of intensive industrialization there is a high degree of chemical pollution of the environment. Different chemical compounds arrive in the environment as products intended for widespread public use or as by products of different technologies. Regardless of their origin, these agents may become factors of possible genetic risk, which may affect the basis of inheritance. Therefore it is imperative that the toxic effects of each chemical substance be determined before it is applied for any purpose.

Since in some way the environment is already saturated with chemical substances, ever more attention is being paid to examining genotoxic effects as well as toxicological studies.

Recently a new group of chemical preparations appeared on the market, namely, chemosterilants intended to control the population of mouse-like rodents whose numbers cause great economic losses. Chemosterilants act by inducing temporary of permanent sterility of one or both sexes in harmful rodents. They may directly or indirectly damage the development or maturation of gametes before or after copulation, prevent the union of egg cell and sperm or prevent implantation of the embryo in the uterus. Thus, chemosterilants may act as chemical agents at different stages of the reproductive cycle (Kincl and Dorfman, 1965; Ericsson et al., 1971; Gao and Short, 1993).

The problem of the potential genetic risk becomes apparent when it is established that many chemicals leave consequences which are not visible immediately but which are exhibited in later generations. Therefore, one may approach this problem from two aspects: a) the damage of rodenticides to all living beings and their teratogenic effects and b) the action of small doses which do not cause visible effects in the threatened organisms but which lead to changes in the genetic base. This may be manifested as difficulties in reproduction, particularly sterility, cessation of embryonic development, poor vitality of offspring at birth or offspring with anomalies.

Since these are few data in tyhe literature about the genotoxic effects of chemosterilants, we have undertaken an investigation of the genotoxic effects of alphachlorhydrin, as a representative substance for this group of rodenticides.

MATERIAL AND METHODS

The genotoxic effects of alphachlorhydrin or U-5897 (3-chloro-1,2-propanediol), which is a highly efficient non-steroid chemosterilant, was investigated in vivo and vitro.

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 alphachlorhydrin per kg cell culture for 2, 3 and 5 days. Mitotic activity and the apperance 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 karyogram.

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.

The results obtained were analyzed by modern statistical procedures according to the Statgraphics 5.0 (Statistical Graphics Corporation, USA) programme.

RESULTS AND DISCUSSION

Considering the results shown in Tables 1 and 2 which refer to the influence of alphachlorhydrin on numeric aberrations (polyploidy and aneuploidy) in mouse bone marrow cells and fibroblasts, it may be seen that the control groups were very uniform and that the relative number of diploid cells was 97.5-100%. Moreover, the level of numeric aberrations was maximally 2.5% which may be regarded as normal because they may be the consequence of the techniques employed. The number of numerical aberrations increased as the dose of alphachlorhydrin was raised. Concerning aneuploid changes, there was always a larger number (twice as much and more) of hypodiploid than hyperdiploid cells, whic may partly be regarded as partial loss of chromosomes during preparation. Although the number of polyploid cells increased during treatment, they were present in much smaller numbers than aneuploid cells. The greatest number (5%) was observed after application of the highest dose, whereas the duration of treatment had little effect. Moreover, the number of numerical aberrations occurring after application of the smallest dose for the shortest time was, on average, very similar to that after maximal exposure. Thus, statistical analysis of the results for numerical aberrations in mouse bone marrow cells and fibroblasts showed no significant differences for either duration of treatment or dose of alphachlorhydrin ($p>0.05$).

Table 1. Cytogenetical effect (numerical changes) of alphachorhydrin on bone marrow cells of the mouse

Number animals	Investigated cells	Number of chromosomes						Aneuploidy		Polyploidy	
		<40	%	40	%	>40	%	X̄	%	X̄	%
Investigated dose 7,5 mg/kg w.b.											
K	69.00	0.00	0.00	69.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Ex 7	83.00	5.71	6.87	74.71	90.01	2.28	2,75	8.00	9.63	1.71	2.06
K	56.0	1.00	1.78	55.00	98.21	0.00	0.00	1.00	1.78	0.00	0.00
Ex 7	93.71	3.86	6.05	58.14	91.25	1.71	2.68	5.57	8.74	2.00	3.14
K	47.00	0.00	0.00	47.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Ex 7	80.57	10.14	12.58	64.43	79.96	6.00	7.45	16.14	20.03	3.43	4.25
Investigated dose 15 mg/kg c.c.											
K	50.00	1.00	2.00	49.00	98.00	0.00	0.00	1.00	2.00	0.00	0.00
Ex 6	52.83	4.00	7.57	46.83	88.65	2.00	3.78	6.00	11.36	1.67	3.15
K	50.00	0.00	0.00	50.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Ex 8	54.37	5.00	9.20	46.75	85.98	2.62	4.82	7.62	14.02	1.62	2.99
K	50.00	0.00	0.00	49.00	98.00	1.00	2.00	1.00	2.00	0.00	0.00
Ex 7	66.28	7.00	10.56	56.43	85.13	2.68	4.31	9.85	14.87	2.14	323
Investigated dose 30 mg/kg c.c.											
K	40.00	1.00	2.50	39.00	97.50	0.00	0.00	1.00	2.50	0.00	0.00
Ex 7	41.00	4.14	10.10	3.50	85.36	1.86	4,53	6.00	14.63	2.14	5.23
K	80.00	1.00	1.25	78.00	97.50	1.00	1.25	2.00	2.50	0.00	0.00
Ex 6	50.67	4.16	6.94	44.50	87.82	2.00	3.94	6.17	12.17	2.00	3.95
K	40.00	0.00	0.00	40.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Ex 8	39.75	3.87	9.73	33.87	85.22	2.00	5.03	5.87	14.78	2.00	5.03

Treatment period 10 days | Treatment period 20 days | Treatment period 30 days

K – Control group Ex – Experimental group

However, the differences between the levels of numerical aberrations in treated groups and the control group were statistically significant ($p<0.01$). Comparing the results obtained in general, it may be seen that alterations in mouse bone marrow cells occurred to a smaller extent than in mouse fibroblasts.

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

Doses	Investigated cells	Number of chromosomes						Aneuploidy		Polyploidy	
		< 40	%	40	%	> 40	%	X̄	%	X̄	%
Treatment period 2 days											
K	68	1	1.47	67	98.53	0	0.00	1.00	1.47	0.00	0.00
I	111	6	5.41	102	91.89	3	2.70	9.00	8.12	1.00	0.90
II	146	28	19.18	106	72.60	12	8.22	40.00	27.40	3.00	2.05
III	112	20	17.86	76	67.86	16	14.28	36.00	32.14	2.00	1.78
Treatment period 3 days											
K	60	0	0.00	60	100.00	0	0.00	0.00	0.00	0.00	0.00
I	102	17	16.67	80	78.43	5	4.90	22.00	21.57	0.00	0.00
II	100	20	20.00	71	71.00	9	9.00	29.00	29.00	2.00	2.00
III	79	11	13.92	51	64.56	9	11.39	20.00	25.32	2.00	2.53
Treatment period 5 days											
K	60	1	1.66	59	98.34	0	0.00	1.00	1.66	0.00	0.00
I	76	12	15.79	51	67.12	3	3.95	15.00	19.74	2.00	2.63
II	62	15	24.19	42	67.74	5	8.06	20.00	32.26	2.00	3.23
III	82	26	31.71	50	60.98	6	7.32	32.00	39.02	4.00	4.88

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

Changes in the structure of chromosomes (structural aberrations) are much more frequent than changes in their number (numerical aberrations).

Structural aberrations may appear in several characteristic forms: gaps (lesions/cracks), fragments (complete breaks) and Robertsonian translocations (joining up of broken pieces). These changes usually occur as a consequence of the influence of genotoxic agents which have the ability to induce alterations in the genetic material. By monitoring these parameters before and after treatment of the test cells with the chosen chemosterilant as a function of dose and time, we wished to determine to what extent it represents a potential danger for genetic material. From the results obtained (Table 3), it can be seen clearly that gaps and fragments appeared sporadically in all the control groups to a maximal level of 1%, while Robertsonian translocations were not registered. There was an evident increase of structural aberrations with dose of alphachlorhydrin and with length of exposure. Thus, a statistically significant difference was found ($p<0.01$) between the effect of the 30 mg/kg and the 7.5 mg/kg

doses on mouse bone marrow calls concerning gaps and fragments. Statistically significant differences between treatments were not established in the other cases either for bone marrow cells or for mouse fibroblast cells ($p>0.05$).

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

Dose	Structural changes (%)		
	Gaps	Fragments	Robertson's translocations
Marrowcells of mouse			
treatment period 10 days			
K	0.00	0.00	0.00
I	4.80	7.08	1.06
II	6.00	6.02	3.02
III	8.53	12.54	4.54
treatment period 20 days			
K	1.00	0.00	0.00
I	7.51	11.04	4.58
II	12.00	14.54	6.04
III	17.03	14.06	7.00
treatment period 30 days			
K	1.00	0.00	0.00
I	8.50	10.04	4.58
II	12.25	16.62	6.04
III	21.04	22.86	7.00
Fibroblast cells			
treatment period 2 days			
K	1.00	1.00	0.00
I	4.02	6.48	2.08
II	3.56	6.96	3.54
III	7.58	10.02	4.46
treatment period 3 days			
K	0.00	0.00	0.00
I	5.55	5.00	3.00
II	7.46	12.54	6.52
II	8.50	15.04	7.96
treatment period 5 days			
K	0.00	0.00	0.00
I	6.04	5.96	6.00
II	7.56	14.04	8.54
III	10.50	17.50	11.00

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 the results obtained it may be seen that alphachlorhydrin treatment can undoubtedly lead to changes in the genetic material, as has been shown by many authors for anticoagulant rodenticides (Capanna et al., 1977; Kataranovski, 1994; Stanimirović et al., 1997). Ciytogenetic changes may lead to numerous undesirable consequences such as genotoxic, mutagenic and teratogenic effects (Baranov, 1971; Hrgovic et al., 1991; Marković, 1996). Of particular importance are alterations which may occur in human and animal embryos. These may be manifested mildly as cessation of embryonal development or seriously as the birth of off spring with anomalies (Elias et al., 1978; Brent; Gao and Short, 1993).

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GENOTOKSIČNI EFEKTI ALFAHLORHIDRRINA KOD LABORATORIJSKOG MIŠA BALB/C SOJA

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SADRŽAJ

U ovom radu su izvršena ispitivanja genotoksičnih svojstava Alfahlorhudrina u različitim dozama 7,5, 15 i 30 mg/kg telesne mase (w.b.), odnosno kulture ćelija (c.c.) u uslovima "in vivo" i "in vitro". U uslovima "in vivo" ispitivanja su uzvršena na ćelijama kostne srži miša tokom eksrozicije od 10, 20, i 30 dana, a u uslovima "in vitro" ka kultiri ćelija fibroblasta miša tokim ekspozicije od 2, 3 i 5 dana.

Procena genotoksičnih efekata, ispitivanih hemosterilanata, je vršena na osnovu numeričkih i strukturnih aberacija na tri eksperimentalne grupe sa po jednom kontrolnom za svaku dozu odnosno ekspoziciju. Na osnovu rezultata naših ispitivanja može se zaključiti da Alfahlorhidrin u testiranim dozama dovodi do genotoksičnih efekata kako na ćelijama kostne srži, tako i na kulturi ćelija fibroblasta miša. Broj numeričkih aberacija sepovećavao sa rastom doze odnosno dužine ekspozicije, međutim statističkom analizom nisu utvrđene signifikantne razlike ($p>0.05$). Analizirajući povećanje broja strukturnih aberacija na ćelijama kostne srži mišau odnosu na doze od 30.0 mg/kg w.b. i 7.5 mg/lg w.b., utvrđene su statistički signifikantne razlike ($p<0.01$). Signifikantne razlike su utvrđene i u pogledu numeričkih i strukturnih aberacija uslovljenih ispitivanim preparatom u odnosu na kontrolne grupe.