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CYTOGENETIC CHANGES IN HUMAN LYMPHOCYTES INDUCED BY ALPHACHLORHYDRINE

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This paper deals with the results of the investigation carried out with regard to genotoxic properties of Alphachlorhydrine administered at doses of 7.5, 15 and 30 mg/kg of cell culture-human lymphocytes, during an exposition time of three days, under in vitro conditions.

The assessment of the genotoxic effects of the tested chemosterilants was performed on the basis of numerical and structural aberrations in three experimental groups, in addition to one control group for each dose.

On the basis of the obtained results it can be conculded that Alphachlorhydrine, administered in the abovementioned doses, induced genotoxic effects on human lymphocyte cells. The number of numeric aberrations increased with a dose increase, and the statistical analysis showed significant differences ($p<0.01$) in relation to the control group, but not between the applied doses ($p<0.05$).

Analysis of the obtained results demonstarted that a dose increase during the same exposition time, resulted in a statistically significant increase ($p<0.01$) of structural aberrations in human lymphocyte cells; the highest level being achieved with the highest administered dose (30 mg/kg c.c.). As for Robertsonian translocations, there was a statistical significance with relation to the control group only in the case of the highest administered dose (30 mg/kg c.c.). Statistically significant differences ($p<0.01$) were recorded between the 30 mg/kg c.c. and 7.5 mg/kg c.c. doses. Also there were 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.

Key words: Alphachlorhydrine, cytogenetic, genotoxic effects, hyman, lymphocytes

INTRODUCTION

The number of populations of rodents has constantly increased during the evolution, causing more and more damages, directly or indirectly, to human populations. Due to their obvious economic, epizootic and epidemiologic importance it is indispensable to undertake some efficient measures in order to

control rodent populations, i.e. to reduce their populations to a safe level so that the health of men, domestic and wild animals is protected. The application of the existing mechanical, physical, biological and chemical methods intended for rodent control, has not yielded the expected satisfactory results, i.e. satisfactory effects have been achieved in smaller buildings, or in less invaded larger buildings.

More and more present rodent resistance to the existing rodenticides requires a constant effort to find new methods or new chemical agents in order to be able to satisfactorily control those rodent populations. Such are ready made baits made on the basis of chemosterilants, Alphachlorhydrine being the most frequently used one. Chemosterilants cause temporary or permanent sterility in these pests, either in both sexes, or individually (Eriksson *et al*., 1971). They can also inhibit, directly or indirectly, the growth and maturation of gametes, prior or after copulation, or prevent the union of the ovum and spermatozoon, as well as the implantation of the ovum in the uterus (Rex, 1970). In addition, they can suppress the sexual development of the young and cause irreversible sterility or death of the young ones due to lactation reduction in their mothers (Gao and Short, 1993).

As it has been proved that some pesticides-rodenticides, produced mostly on the basis of derivates of cumarin and INANDION (Soldatović *et al*.,1979, 1980, 1981; Kataranovski, 1985; Sofradžija *et al*., 1989; Vučinić, 1994; Stanimirović *et al*., 1997) cause cytological, mitotic and chromosomal aberrations, and that the available literature contains scanty data on genotoxic effects of chemosterilants, we undertook to investigate whether Alphachlorhydrine can induce genotoxic effects on human lymphocyte cells.

MATERIAL AND METHODS

The genotoxic effects of Alphachlorhydrine s. U-5897 (3-chloro-1,2-propandiol) a highly efficient nonsteroid chemosterilant, were investigated *in vitro*.

For the studies, human lymphocites were treated with 7.5, 15 and 30 mg Alphachlorhydrine per kg cell culture during exposure of 3 days. Mitotic activity and the appearance of structural and numerical aberrations were investigated. 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).

Cell culture humane lymphocytes were prepared by the method Evans and O'Riordan (1977).

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

RESULTS ADN DISCUSSION

Table 1 contains the obtained results pertaining to the effect of Alphachlorhydrine (administered at doses of 7.5, 15 and 30 mg/kg c.c.) on human lymphocytes under *in vitro* conditions from the point of numerical aberrations. During the three-day treatment three experimental and one control

group of cells were monitored. From 78 to 102 metaphase figures were examined in each experimental group, and 84 metaphase figures in the control group.

Table 1. Numerical aberration in human lymphocytes induced by Alphachlorhydrine

Doses	Investigated cells	Number chromosomes						Aneuploidy		Polyploidy	
		<46	%	46	%	>46	%	Number	%	Number	%
K	84	2	2.38	82	97.61	0	0.00	2	2.38	0	0.00
I	102	8	7.84	88	86.27	6	5.88	14	13.72	0	0.00
II	78	10	12.82	60	76.92	6	7.69	16	20.51	2	2.56
III	94	21	22.34	61	64.89	9	9.57	30	31.91	3	3.19

Investigated doses: K – Control group; I – 7.5 mg/kg c.c.; II – 15 mg/kg c.c.; III – 30 mg/kg c.

The diploid number of chromosomes in the control lymphocyte group reached 97.61%, in aneuploid cells 2.38%; whereas poliploid cells were not registered. The dose increase was accompanied with a decrease in the number of diploid cells – the lowest value of 64.89% being attained with the highest dose of 30 mg/kg c.c. At the same time, the number of aneuploid cells was markedly increased at the highest dose, the attained level reaching 31.91% of which the greatest part belonged to haploid cells (22.34%). An increase in the dose, i.e. at the doses of 15 mg and 30 mg/kg c.c. occurred an increase in the number of poliploid cells, up to the level of 2.56 - 3.19%. This increase as a function of dose was statistically significant ($p<0.01$). The incidences of changes in all the three applied doses in relation to the control group were also statistically significant ($p<0.01$), except in the case of poliploidy when no significant statistical difference was recorded ($p<0.05$).

The second part of the experiment refers to the effect of various doses (7.5 15 and 30 mg/kg c.c.) of Alphachlorhydrine on human lymphocyte cells during the three experimental days with regard to the occurence of structural aberrations. Table 2 contains the results at the level of average mean values.

Table 2. Cytogenetical effects (structural changes) of Alphachlorhydrine on humans lymphocites (average values)

Group	Structural changes (%)		
	Gaps	Fragments	Robertson's Translocations
K	0.0	1.0	0.0
I	3.5	6.5	0.0
II	8.0	10.5	0.0
III	10.5	12.5	2.0

Investigated doses: K – Control group; I – 7.5 mg/kg c.c.; II – 15 mg/kg c.c.; III – 30 mg/kg c.c

Each experiment was conducted on three experimental groups and one control group. Within each group, on the average 100 metaphase figures were examined. In the control groups gaps and fragments were recorded up to the level of 1%; whereas Robertsonian translocations were not recorded. A dose increase, during the same exposition time, is accompanied with an increase in values of structural aberrations; the highest level being reached at the highest administered dose (30 mg/kg c.c.). This increase as a function of dose was statistically significant ($p<0.01$), except in the case of Robertsonian translocation where the statistical significance with regard to the control group was recorded only in the case of the highest administered dose (30 mg/kg c.c.). Statistically significant differences ($p<0.01$) were recorded between the 30 mg/kg c.c. and 7.5 mg/kg c.c. doses. Also, statistically significant differences between the levels of gaps and fragments, in comparison to Robertsonian translocations ($p<0.01$) were recorded, while the numbers of gaps and fragments were not significantly different.

On the basis of the obtained results it can be concluded that Alfahlorhidirin in administered doses, during an exposition time of three days, can bring about damages to the genetic material. Cytogenetic changes were recorded also in other rodenticides (Sofradžija *et al*., 1989; Kataranovski, 1994; Stanimirović *et al*., 1997; Teodorović *et al.*, 1999; Teodorović, 2004), as in many other substances used in veterinary medicine (Albertini *et al*., 2000; Stanimorović *et al*., 2003; Platet *et al*., 2004; Đelić *et al*., 2006). The majority of these investigations, similar to our experiment, was performed under *in vitro* conditions on human leucocyte cell cultures. Alphachlorhydrine, in addition to its genotoxic effects on genetic material, induces sterilization of male rats, temporary sterilization of guinea pigs and monkeys (Ericsson and Baker, 1970; Kirton *et al.*, 1970; Ericsson *et al*., 1971) and has no effect on fertility of mice and rabbits (Ericsson, 1970).

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CITOGENETIČKE PROMENE LIMFOCITA ČOVEKA INDUKOVANE ALFAHLORHIDRINOM

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SADRŽAJ

U ovom radu su izvršena ispitivanja genotoksičnih svojstava Alfahlorhidrina u različitim dozama: 7.5, 15 i 30 mg/kg kulture ćelija – limfocita čoveka, tokom ekspozicije od 3 dana, u uslovima "*in vitro*".

Procena genotoksičnih efekata, ispitivanih hemosterilanata, je vršena na osnovu numeričkih i strukturnih aberacija na tri eksperimentalne grupe sa po jednom kontrolnom za svaku dozu.

Na osnovu rezultata naših ispitivanja može se zaključiti da Alfahlorhidrin u testiranim dozama dovodi do genotoksičnih efekata na ćelijama limfocita čoveka. Broj numeričkih aberacija se povećavao sa rastom doze i statističkom analizom su utvrđene signifikantne razlike ($p<0,01$) u odnosu na kontrolne grupe, ali ne i između primenjenih doza ($p>0,05$). U slučaju poliploidija nije utvrđena statistički značajna razlika u odnosu na kontrolnu grupu ($p>0,05$).

Analizirajući dobijene rezultate utvrđeno je da sa povećanjem doze pri istoj ekspoziciji dolazi do statistički značajnog ($p<0,01$) povećanja broja strukturnih aberacija na ćelijama limfocita čoveka, pri čemu najviši nivo je postignut kod najviše primenjene doze (30 mg/kg c.c.). Kada su u pitanju Robertsonove translokacije statistička značajnost u odnosu na kontrolnu grupu utvrđena je tek kod najviše primenjene doze (30 mg/kg c.c.). Statistički značajne razlike ($p<0,01$) su zabeležene između doza od 30 mg/kg c.c i 7,5 mg c.c. Takođe statistički značajne razlike između nivoa otvora i fragmenata mogu se uporediti sa Robertsonovim translokacijama ($p<0,01$), dok se broj otvora fragmenata neće značajno promeniti.