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STUDY OF GENOTOXICITY OF ENVIRONMENTAL CHEMICAL POLLUTION ON TWO SPECIES OF GENUS *APODEMUS (RODENTIA MAMMALIA)*

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Abstract

In present work, genotoxicological effects of chemical pollution of an industrial region, on species of genus *Apodemus (Rodentia, Mammalia)*, were studied. The two species, were *A.flavicolis* and *A.agrarius*. In this study chromosomal aberrations of bone marrow cells were analysed and were compared according to sex and different species in polluted and unpolluted (control) areas. Results showed that frequencies of chromosomal aberrations, including breaks, between two groups were statistically significant ($P<0.005$); and also sensitivity of two sexes and two species against chemical pollutant, were not significant.

Introduction

Historically, geneticists tried to investigate the mutagenic effects of ionizing radiations before the effects of chemicals.

We are daily exposed to literally hundreds of chemical agents in perhaps staggering numbers of combinations via the air, water and food, by skin contact, medication, in industrial and indoor environments and lifestyle.

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Hundreds of investigators, worked on genotoxic effects of chemicals on different organisms (13,17,18,19,20,24), also, numerous investigators worked on the effects of pollutants on ecosystems (4,7).

There are several ways to study genetic hazards of chemical pullotion and evaluate its genotoxicity on man. One of the usual ways, is to study its effects on animals, especially rodents. The genus which was used in present study was wood mice, *"Apodemus"*. Sensitivity potential aginst environmental chemical pullotants among two species of genus *Apodemus, A.flavicolis* and *A.agrarius*, were also studied in this work.

Present work will determine and compare sensitivitiy of these two species against chemical pollution and will try to look in this view to find another reason for wider spread of *A.flavicollis* than *A.agrarius*. This study showed that the chemical pollution of environment cause chromosomal aberration. Also it seemed that there is no difference between sensitivity of *Apodemus agrarius* and *A. flavicollis* against chemical pollution. How about the Man? Is he more sensitive than these small mammals or not? The future works will answer this very important question and also will search the danger of the chemical hazards to the " gene pool of man".

Materials and methods

Pancevo (Panchevo), and Jastrebac (Yastrebats), were selected as research fields, in which we collected our subjects. The first one, is an old industrial zone very close to Belgrade (capital city of Yugoslavia), and the Jastrebac, is an non industrial area near Belgrade. Both areas have rather the same climatological and geographical features. Based on the data that were avilable in the Department of Climatology , the mean values of concentrations of most important pollutants of Pancevo were more than standard values.

In October 1992, Longworth live-traps, baited with sardin were used to capture rodents. The studies were carried out in two study areas, and Pancevo as polluted area (P) Jastrebac, as an unpolluted, control area (C). In each area, the traps were placed for three days and were examined every morning (8:30 A.M)

to collect live animals, and rebatting. The captured animals were individually marked by putting them in separate cages. Animals other than *Apodemus*, if captured , were not analysed in present work. The procedure of chromosome analysis were done using standard technique.

Metaphase analysis were carried out only by one person, and then checked the aberrations by another person. The metaphases that were shown as morphologically well defined, as well as being without superposed chromosomes, were selected for the analysis. At the first step fifty metaphases from each animal were analyzed and registered as normal or as displaying chromosome aberrations. Aberrations considered were chromatid and chromosome breaks, gaps, acentric fragments, centric fusion, rings and etc. Identification of aberration was based upon ISCN (11,21,22).

The statistical analysis was based on: i. "Gap per cell" , ii. "Break per cell", iii."Other aberrations per cell", iv. "no. of abr. with Gap", v. "no. of abr. without Gap", vi. "frequency of cells with abr. + Gap", vii. "frequency of cells with abr. - Gap". All groups were compared with each other, according to these seven factors. The significance of differences between samples, was tested by using t-test. The steps of analyses were as follow:

1 st step: males and females were compared, among each group, separately:

(a). If there was no significant difference between two sexes, should pool their data and go to the next step; and (b). if there were sig. differences, should go to the next step for each sex, separately.

2nd step: In 2nd step, different groups were compared with each other.

If significent differences were not observed between PI and PII in one hand, and CI and CII in the other hand, should go to the next step.

3rd step: PI + PII x CI + CII, Animals collected from Panchevo, were compared with Animals collected from Jastrebac.

Results

Table 1, shows the animal groups and their codes, according to different collecting areas.

Table 2, shows the number of cells that scored and number of their chromosomal aberrations, according to the species and sex of the animals which captured in Panchevo (polluted area), and Jastrebac (unpolluted area). Because the frequencies of the aberrations such as translocations, rings, dicentric fragments, etc. were low, they were pooled and presented as "others".

Table 3 , shows results obtained in different stages of all three steps of statistical analyses.

Discussion

The cytogenetic monitoring of wild rodent has been used as a method to evaluated the possible clastogenic effect of chemical environmental pollutants produced by industrial factories. This type of approach-cytogenetic monitoring of natural populations - though complex, may provide more information on clastogenic effects on a given environment than isolated in vivo analysis , or laboratory in vitro ones using rodent strains bred for this finalitt (6).

The realisation that certain man-made chemicals and some naturally occurring agents in the man's environment induce cancer, has provided impetus to the genetic toxicology (toxicogenetics). Evaluation of safety of therapeutic agents employing somatic and meiotic cells of mammals has some significance in human health risk assessment (2). Mutagenecity is considered as an accepted toxicological parameter for such analyses (3). Bone marrow chromosomal study has been recommended as an ideal protocol among the available cytogenetic assays to screen potential mutagenic effects of environmental agents (16,14). In our study areas human were living as well as rodents. Therefor, it deserves to elucidate whether chemical pollutants in industrial areas are able to produce chromosomal aberrations in human.

The real significant differences between exposed groups (PI & PII) and control groups (CI & CII), are due to breaks and other aberrations but not to gap.

The significance of gaps as an expression of genetic damage is not yet fully understood (9,10). Counted them separatly but not included in the categories of true aberrations. However gaps and fragments as "light" aberrations in monkey lymphocyte chromosome analysis was recorded (8).

Some works on human lymphocyte chromosomes (23), showed the gaps but not regarded them as aberrations, but others (15) in their studies accounted them as indicators of genotoxic potential of reference compounds; while in our work although frequencies of gaps in PI and CI as well PII and CII were different, they were not statistically significant.

In this study sensitivity of two species were not significantly different, but the two sets of data showed a mild difference. It is known that different species, or even strains, submitted in a given way to the same type, quantity, and concentration of a clastogenic agent may react differently , since their metabolism differs (1,5,12). In a natural population, it is generally not known if all species are being submitted to the same pollutants, in the same quantity, and/or in the same way, and what quantity of these substances and/or their metabolites reach the animal's bone marrow. It can only be informed that these species are reacting differently to environmental impact. For this type of study , these observations stress the importance of undertaking intraspecific comparisons.

In a similar study in south Brazil (6) the researchers noted that "finding organisms in a given environment had present a greater number of microscopically detectable mutations may point to mutagenic agents provoking such alterations, inefficient repair mechanisms, affected cells not being eliminated, and increasing genetic load for the population , since organisms carrying these alterations are surviving. The damage described here might nontheless be just a fraction of the total damage. Other cells and/or organisms carrying mutations which died as a consequence, will not be detected .

The results which are summarized in table 3, shows chronic treatment of industrial chemical pollutants, in our research area, induce somatic chromosome anomalies in both species of genus *Apodemus*, that we examined. Also the result

shows , that in our work , there are no significant differences between sensitivity of males and females of both species.

The increased frequencey of chromosomal aberrations, for total cell number containing chromosome aberrations as well as for total analyzed chromosomes, found in polluted animals can be interpreted as one of the effects of the environmental pollutants to which the animals are exposed.

The recent concern for chemically induced mutations and the development of practical methods for determining mutagenic activity, accents the need for a close alliance between the diverse science of toxicology and genetics. The problem of selection of hybrid or inbred strains of experimental animals, the interpretation of results from reproductive, carcinogenecity, or teratology studies surely requires the expert knowledge of both toxicologists and geneticists, and their cooperative participation is long overdue.

Not only should geneticists become actively involved in the development of screening and determining safety testing protocols, but a decided effort should be made to insure their participation in expert committees concerning policy, protocol, or vital decisions about the possible deleterious effects of environmental agents.

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Table 1- Animal groups and their codes, according to different collecting areas.

groups		species	collecting areas	
no	code		name	situation
I	PI	A.flavicolis	Pancevo	polluted
II	PII	A.agrarius	Pancevo	polluted
III	CI	A.flavicolis	Jastrebac	unpolluted
IV	CII	A.agrarius	Jastrebac	unpolluted

Table 2- Number of cells that scored and their chromosomal aberrations, according to the species and sex of the animals which captured in two research areas.

codes	species	sex	no.of cells	no. of cells with			no. of cells with	
			scord	Gaps	Breaks	Others	ABS+G	ABS-G
PI	A.flavicolis	Total	1384	34	37	65	136	102
		Male	565	12	16	22	50	38
		Female	819	22	21	43	86	64
PII	A.agrarius	Total	1689	36	52	71	159	123
		Male	1014	16	33	43	92	76
		Female	675	20	19	28	67	47
CI	A.flavicolis	Total	932	15	6	11	32	17
		Male	519	6	4	5	15	9
		Female	413	9	2	6	17	8
CII	A.agrarius	Total	550	4	11	5	20	16
		Male	350	3	8	5	16	13
		Female	200	1	3	0	4	3

ABS+G, stands for total of different types of chromosomal aberrations plus gaps; ABS-G, stands for total of different types of chromosomal aberrations minus gaps.

Table 3- Summary of results obtained in different steps.

steps	stages	X1	X2	X3	X4	X5	X6	X7
First	PI_m x PI_f	-	-	-	-	-	-	-
"	PII_m x PII_f	-	-	-	-	-	-	-
"	CI_m x CI_f	-	-	-	-	-	-	-
"	CII_m x CII_f	-	-	0	-	-	-	-
Second	PI x CI	-	+	+	+	+	+	+
"	PII x CII	+	+	+	+	+	+	+
"	PI x PII	-	-	-	-	-	-	-
"	CI x CII	-	-	-	-	-	-	-
Third	PI+PII x CI+CII	-	+	+	+	+	+	+

- : no significant difference, + : significant difference, 0 : t-test was not possible.

X1: Gap per cell= No. of Gaps/No. of cells scored

X2: Break per cell= No. of Breaks/No. of cells scored

X3: Other abr. per cell= No. of other abr. /No. of cells scored

X4: No. of abr. with gap= No. of Gaps+No. of Breaks+no of Other abr.

X5: No. of abr. without gap= No. of Breaks+No. of Other abr.

X6: Frequency of cells with abr.+Gap= (No. of cells with Gap+No. of cells with Break+No. of cells with Other abr.)/No. of cells scored.

X7: Frequency of cells with abr.-Gap=(No. of cells with Break+No. of cells with Other abr.)/No. of cells scored.

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