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THE STUDY OF CYTOGENETIC EFFECT OF ACTELLIC INSECTICIDE IN BONE MARROW CELLS OF MICE AFTER SINGLE EXPOSURE AT HIGH TEMPERATURE

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ABSTRACT

We aimed to investigate in detail the structural mutations after influence of actellic insecticide at different doses and different temperature conditions. We tested effects of single introduction of different doses of actellic: 48, 96, 192, and 384 mg/kg. Cytogenetic effect of actellic in bone marrow cells at different temperature regimens was studied. Following actellic administration, animals were being in two temperature regimes: normal (18-20°C) and high (37-38°C). After treatment with actellic, animals were kept within 1, 2, 4 and 8 hours at high temperature (37-38°C), i.e. in a thermostat (TV-80) with access of air. In experiments, the animals were divided into seven groups. The animals of the 1st group were administered actellic at a dose 384 mg/kg and kept under normal temperature until slaughtering. The animals of the 2nd group after administration of actellic were immediately placed in an environment with a temperature of 37-38°C. Animals of the 3rd, 4th, 5th, and 6th groups after treatment with actellic were transferred to conditions with high temperature, respectively, for 1, 2, 4 and 8 hours. The 7th group of mice was under normal temperature conditions without treatment with actellic. Our results have shown that in normal temperature conditions single exposure of actellic (384 mg/kg) did not affect the genetic apparatus of somatic cell, evidenced by the frequency of chromosome aberrations in bone marrow cells that were within the control levels. However, at high temperatures (37-38°C) the pesticide caused 6-fold larger number of chromosome aberrations in bone marrow cells, in comparison with normal conditions.

UDC CODE & KEYWORDS

■ UDC: 616.899.65-632.91- 612.419 ■ Actellic ■ Cytogeneti effect ■ Chromosome aberrations ■ Bone marrow cells ■ Mice

INTRODUCTION

As Tiunov (1986) and Prozorovsky et al. (1997) reported, the intensive use of pesticides in agriculture leads to increase of contingent of the population who has direct contact with them in the manufacturing and cornfield conditions. Entering the human body with foodstuffs of plant origin, pesticides and their metabolites accumulate in the body, affecting the health (Blain, 1990; Breslin, 1996). Pesticides are mainly (85%) enter the body with food and can accumulate in various organs (Yermolov et al., 1989; Kagan, 1990). Pesticide were revealed in the blood of pregnant women, breast milk, and also in placenta (Kaloyanova-Simeonova, 1980; Kagan, 1984). Depending on the physico-chemical properties, resistance to physical and chemical influences, solubility in water and organic acids, the rate of metabolism in humans and animals, as well as formation of metabolites, pesticides may have different effects on human health and the gene pool of the population (Sokolova, 1990; Saprin, 1991; Borisenko et al., 1992; Kontush, 1992; Karpenko et al., 1993; Volonyanskaya et al., 1994; Melnikov et al., 1995). Borisenko et al. (1992) established a direct correlation between the intensity of pesticide use and health outcomes in the population of rural areas.

Many pesticides are mutagenic, therefore, mutagenic stress on populations of plants, animals and humans has catastrophically increased (Kurinny et al., 1976; Kulygina, 1984). According to studies by Sidorenko (1978), Tashkhodjaev et al. (2006) and Kurbanov et al. (2008), 75% of mutations in humans is caused by chemicals, including pesticides.

Phosphorus organic compound (POC) is one of the most important classes of modern pesticides. Among POC, actellic insecticide has broad spectrum of activity and retains the biological activity from few weeks till several months, depending on the application conditions. There are no published data on the influence of actellic on the genetic apparatus of mammals. In this regard, we have aimed to investigate in detail the structural mutations after influence of actellic insecticide at different doses and different temperature conditions.

Materials and methods

Actellic is produced as a 50% emulsion concentrate by “Zeneca - Plant Protection”, UK. The drug is highly soluble in most organic solvents; solubility in water is 5 mg/l; destroyed by acids and alkalis; stable in aqueous solutions with a neutral reaction. Actellic belongs to moderate hazardous substances of class III; the mean lethal dose at oral administration for rats is 2050 mg/kg, for white mice is 1180 mg/kg (Kaloyanova-Simeonova, 1980).

We tested effects of single introduction of different doses of actellic: 48, 96, 192, and 384 mg/kg. Cytogenetic effect of actellic in bone marrow cells at different temperature regimens was studied. Following actellic administration, animals were being in two temperature regimes: normal (18-20°C) and high (37-38°C). After treatment with actellic, animals were kept within 1, 2, 4 and 8 hours at high temperature (37-38°C), i.e. in a thermostat (TV-80) with access of air.

In experiments, the animals were divided into seven groups. The animals of the 1st group were administered actellic at a dose 384 mg/kg and kept under normal temperature until slaughtering. The animals of the 2nd group after administration of actellic were immediately placed in an environment with a temperature of 37-38°C. Animals of the 3rd, 4th, 5th, and 6th

groups after treatment with actellic were transferred to conditions with high temperature, respectively, for 1, 2, 4 and 8 hours. The 7th group of mice was under normal temperature conditions without treatment with actellic.

In all experiments, for cytogenetic analysis of bone marrow cells, animals were sacrificed after 24 hours after treatment with actellic. To account for chromosome aberrations in metaphase preparations of bone marrow cells were prepared by the standard technique (Guidelines for the hygienic evaluation of new pesticides, Kiev, 1988, p.55-89). Chromosome preparations were analyzed on a microscope MBI-6 with immersion lens of 90x. For the analysis, we selected metaphasal slabs, in which all chromosomes were allocated separately from each other. The possible types of chromosome restructurings detected in metaphase were analyzed. The frequency of mutations of chromosomes in bone marrow was determined by the number of cells with chromosome rearrangements. When considering chromosome rearrangements, in each variant of the experiment we used at least six animals and analyzed 300-1500 metaphases. The incidence of metaphases with rearrangements (% of metaphases with chromosome aberrations) and the frequency of aberrations (relative number of aberrations for 100 metaphases) were determined. Digital data were processed by the standard statistical methods.

Results and discussion

Table 1 demonstrates the data on the frequency of chromosome aberrations in bone marrow cells of mice after treatment with different doses of actellic at normal temperature 18-20°C.

Table 1: The frequency of chromosome aberrations in bone marrow cells of mice after single effect of different doses of actellic at temperature of 18-20°C

Dose, mg/kg	Number of investigated		Metaphases with aberrations		Aberrations	
	Animals	Metaphases	Number	%	Total	For 100 metaphases
Control	11	1040	5	0.48	5	0.48
48	9	1036	7	0.61	7	0.61
96	13	942	6	0.63	6	0.63
192	10	1020	5	0.49	5	0.49
384	12	890	6	0.67	6	0.67
768	11	956	28	2.92***	32	3.34***
1000	9	979	29	2.96***	33	3.37***
Note : * - significant differences to control group (***-P<0.001)						

Source: Author

We studied cytogenetic activity of actellic exposure at doses 48, 96, 192, 384 and 768 mg/kg, respectively 1/25, 1/12, 1/6, and 1/3 close to LD50. Our results show that low-dose of actellic does not affect the genetic apparatus of cells in the bone marrow of mice. In this regard, the frequency of chromosomal aberrations after introducing doses 48, 96, 192 and 384 mg/kg remained approximately at the control level of 0.61 0.63, 0.49 and 0.67%, respectively. The number of chromosome aberrations significantly increased after introduction high doses of the preparation. Thus, at a dose of 768 mg/kg it increased 6-fold. However, we did not observed increasing the number of chromosome rearrangements upon further increase actellic doses, and even a higher dose as 1000 mg/kg caused a similar increase of number of chromosome rearrangements (2.96%) like the dose 768 mg/kg (2.92%) (Table 1).

Table 2: The frequency of chromosome aberrations in bone marrow cells of mice after single exposure of different doses of actellic at temperature of 37-38°C

Dose, mg/kg	Number of investigated		Metaphases with aberrations		Aberrations	
	Animals	Metaphases	Number	%	Total	For 100 metaphases
Control	7	964	5	0.51	5	0.51
48	9	929	5	0.53	5	0.53
96	8	971	6	0.61	6	0.61
192	10	1038	17	1.63*	17	1.69*
384	9	1036	33	3.18***	37	3.57***
768	10	1141	37	3.24***	42	3.68***
Note : * - significant differences to control group (*-P<0.05, ***-P<0.001)						

Source: Author

In bone marrow cells of animals, which were placed in conditions with high temperatures (37-38°C) immediately after administration of actellic at doses 48 and 96 mg/kg, the frequency of chromosome aberrations remained roughly at the control levels 0.53 and 0.61%, respectively. However, after actellic had exposured at higher doses as 192 and 384 mg/kg, and the animals had been placed in conditions of high temperature (37-38°C), chromosomal aberrations in bone marrow cells increased. Thus, actellic at doses of 192 and 384 mg/kg at normal temperature (18-20°C) did not cause chromosomal mutations in bone marrow cells of mice. Later on, in experiments with the similar doses of actellic, after the animals had been placed in conditions with high temperature (37-38°C), the frequency of chromosome aberrations increased 3-fold (1.63%) and 6-fold (3.18%), respectively, in comparison with control (0.51%). A higher dose of actellic as 768 mg/kg did not increase the number of chromosomes mutations (3.24%), compared to the dose of 384 mg/kg (3.18%). In this regard, the frequency of chromosome aberrations, and the number of cells per 100 metaphases were the same as in the dose of 384 mg/kg (Table 2).

Table 3 illustrates the data on the frequency of chromosome aberrations in the bone marrow cells of mice after single exposure of actellic, depending on the temperature regimen.

Actellic at a dose of 384 mg/kg had no cytogenetic effect on animals' cells at normal temperature conditions (18-20°C) and the frequency of the studied chromosomes (0.50%) and the number of cells per 100 metaphases (0.5) remained at control levels. In bone marrow cells of animals, which were placed in conditions with high temperatures (37-38°C) immediately after administration of actellic, the frequency of chromosome aberrations (2.65%) and the number of metaphases per 100 cells (2.90) was increased 5-fold, compared with experiments done at normal temperature (0.5%) (Table 3).

Table 3: The frequency of chromosome aberrations in bone marrow cells of mice after single effect of actellic (384 mg/kg), depending on temperature regimes

Dose, mg/kg	Number of investigated		Metaphases with aberrations		Aberrations	
	Animals	Metaphases	Number	%	Total	For 100 metaphases
Control, 18-20°C	9	982	5	0.50	5	0.50
Actellic, 18-20°C	11	1126	6	0.53	6	0.53
Actellic immed., 37-38°C	9	1204	32	2.65***	35	2.90***
Actellic after 1 h, 37-38°C	10	976	9	0.92	9	0.92
Actellic after 2 h, 37-38°C	9	920	6	0.65	6	0.65
Actellic after 4 h, 37-38°C	10	996	5	0.50	5	0.50
Actellic after 8 h, 37-38°C	8	871	5	0.45	5	0.45
Note : * - significant differences to control group (***-P<0.001)						

Source: Author

After influence of high temperature, in mice within an hour after actellic injection chromosome aberrations (0.92%) were almost 3-fold less than that in the experiment where the animals were placed immediately in conditions with high temperatures (37-38°C) after actellic administration. In

high temperature (37-38°C) conditions, after two hours of experiment, frequency of chromosomal aberrations (0.65%) was not different from control levels (0.50%). At 4 and 8 hours after actellic administration, the frequency of chromosome aberrations (0.50 and 0.45 %, respectively) remained at control levels (Table 3).

Thus, in normal temperature conditions single exposure of actellic (384 mg/kg) did not affect the genetic apparatus of somatic cell, evidenced by the frequency of chromosome aberrations in bone marrow cells that were within the control levels. However, at high temperatures (37-38°C) the pesticide caused 6-fold larger number of chromosome aberrations in bone marrow cells, in comparison with normal conditions. We assume that increase in the frequency of structural chromosome mutations at high temperature can be explained by the fact that under normal conditions the potential changes that occur at actellic exposure do not transfer to structural mutations and are restored, and at high temperature part of them transforms to mutations.

Conclusion

Thus, qualitative and quantitative analysis has shown that according to test of accounting chromosome aberrations in bone marrow cells of mice, by mutagenic activity actellic should be attributed to moderately hazardous pesticides.

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