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The Cytogenetic Effects of Logran on Bone Marrow Cells of *Mus musculus*

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Abstract: The cytogenetics effects of Logran, active substance Triasulfuron, at different concentrations, sex and application periods were investigated on bone marrow chromosomes in mice. Male and female mice were applied intraperitonally with 125, 250 and 500 μg mL^{-1} concentrations of Logran for 12 and 24 h. Logran induced chromosome aberrations like chromatid gap, isochromatid gap, chromatid break, isochromatid break and centromeric attenuation on bone marrow cells of mice. Increase of chromosomal aberration reduced mitotic activity. According to the our study, Logran is genotoxic on bone marrow cells of *Mus musculus*.

Key words: Logran, chromosome aberration, bone marrow, sulphonylurea

INTRODUCTION

Sulphonylurea herbicides are characterised by low field rates, high herbicidal activity, broad action spectrum, good crop selectivity and low human and animal toxicity. Their mode of action is highly specific since these herbicides inhibit acetolactate synthase (ALS), an enzyme of the biosynthetic pathway of the branched amino acids (valine, isoleucine and leucine) in plants and microorganisms (Zanardini *et al*., 2002). Inhibition of ALS causes very rapid cessation of growth and inhibition of cell division and cell elongation due to lack of essential amino acids needed for protein synthesis. They are a very important group of herbicides because of their effectiveness against a large number of weeds, good crop and activity at low concentrations (Wilhelm and Hollaway, 1998).

Logran is one of sulphonylurea herbicide and its active substance is Triasulfuron. This herbicide is using in agricultural area in Turkish Thrace. Large amounts usage of sulphonylurea herbicides can cause accumulation in soil and water. These chemicals are contaminated to living organisms directly or with accumulation of them in food. Effects of chemical substances should be investigated on living organisms before use. Among more than 200 known bioassays in the literature (Waters *et al*., 1990). One of these tests is the effect of the chemical on mitotic cell division. The genotoxic effects of herbicides have been investigated on animals and human (Galloway *et al*., 1998; Marks, 2000; Gajanayake, 2002). But it is not found any report about genotoxic effects of Logran on animals. In the present study, it is aimed to investigate cytogenetics effects of Logran on bone marrow of *Mus musculus*.

MATERIALS AND METHODS

Logran of which effective substance is Triasulfuron was used as a test substance (Novartis AG., Basle, Switzerland).

Eighty adult male and female *Mus musculus* (20-25 g) obtained from Istanbul University Scientific Research Center. Twenty mice were used as a control group and sixty mice were used as application group. Animals were maintained on food and water *ad libitum*.

Four groups were prepared for the experiment (5 animals each). Three of these were experiment groups. One of these was the control group. Initially, an LD_{50} for the herbicide was determined. One-eight, one-fourth and half of LD_{50} were selected for the genotoxic assay (125, 250, 500 μg mL^{-1}, respectively). Male and female mice were applied intraperitonally with 125, 250 and 500 μg mL^{-1} concentrations of Logran, dissolved in distilled water, for 12 and 24 h (0.01 mL g^{-1}). Distilled water was given to control group during these application periods.

For each concentration, five animals were used. Bone marrow chromosomes were prepared according to the method of Preston *et al*. (1987). Colchicine (0.01%), used to arrest the cells at metaphase, was injected intraperitoneally (0.01 mL g^{-1}) 3 h before servical dislocation. Then the bone marrow from a femur was flushed out in 1% sodium citrate, the cells were collected by centrifugation 5 min at 1000 rpm. One percent sodium citrate was used as hypotonic solution and the cells were incubated at 37°C for 25 min and fixed in 1:3 acetic acid:methanol. This fixation step was repeated until the lymphocytes were turned to white. All dried preparations were made and the slides were stained with 10% Giemsa in Sörensen buffer for 10 min.

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Hundred well spread metaphase were scored for each concentration and application period. Chromosomal aberrations were investigated.

For mitotic index of each concentrations, 3000 cells were scored. Dividing cell number were divided by total cell number for calculating the percentage of dividing cells.

The results were statistically analyzed using Chi-Square for chromosomal abnormalities and t-student test for mitotic index. Each treatment group was compared with control.

RESULTS

In present study, chromosome aberrations were observed like chromatid gap, chromatid break, isochromatid gap, isochromatid break and centromeric attenuation. Samples of the most frequent type of abnormality observed were given in Fig. 1 and 2.

The results of obtained from treated and control group were given in Table 1. All of the concentrations of Logran induced mitotic division abnormalities but these abnormalities except centromeric attenuation weren't significant when compared with control. All of the concentrations of Logran induced significant ($p \leq 0.001$) centromeric attenuation in both male and female mice for 12 h application period. As for 24 h application period, centromeric attenuation was increased significantly ($p \leq 0.001$) at 125 μg mL^{-1} Logran in female and at 250 and 500 μg mL^{-1} Logran in male mice.

The total number of chromosome aberrations of 12 h application period was compared to the total number of chromosome aberrations of 24 h application period. A significant difference was observed between these 2 application periods. Centromeric attenuation and chromatid gaps significantly increased at 12 h application period when compared with 24 h application period. The result showed that male and female mice were affected at 12 h application period more than 24 h application period.

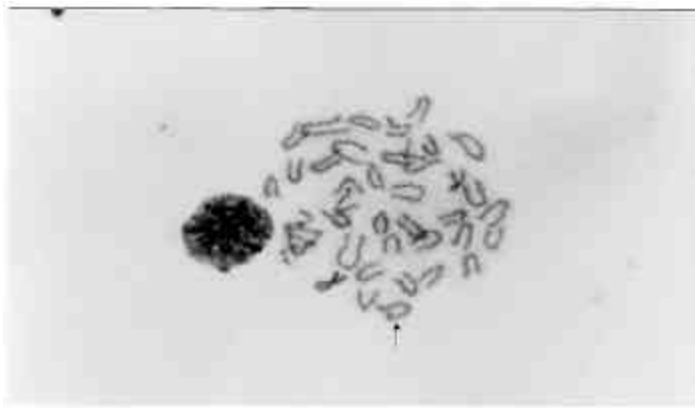

Fig. 1: Chromatid break observed after 500 μg mL^{-1} Logran application for 12 h

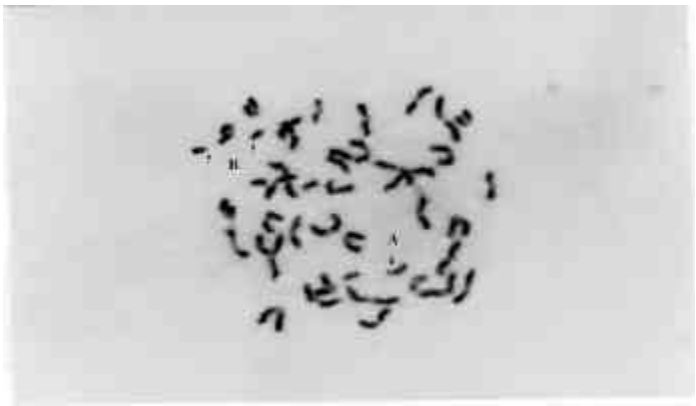

Fig. 2: Aberrations observed after 250 μg mL^{-1} Logran application for 12 h, A) Chromatid gap, B) Centromeric attenuation

Table 1: Effect of Logran on mice bone marrow

A.P and Sexes	Conc. ($\mu g\ mL^{-1}$)	OMN	NMN	NCA (+gap)	CG	IG	CB	IB	CA	MI
12 h ♀	Control	100	92	8	1	-	2	-	5	2.86
	125	100	37	63	8	1	3	1	50***	2.26
	250	100	47	53	5	1	2	-	45***	1.46***
	500	100	48	52	9	2	2	-	39***	1.06***
12 h ♂	Control	100	94	6	1	-	1	-	4	3.26
	125	100	57	43	9	3	2	-	29***	2.92
	250	100	60	40	7	1	2	1	29***	1.86*
	500	100	36	64	7	1	6	-	50***	1.60***
24 h ♀	Control	100	92	8	2	-	3	-	3	3.40
	125	100	76	24	1	1	1	-	21***	2.80
	250	100	85	15	4	-	1	-	10	3.30
	500	100	88	12	4	-	-	-	8	2.15*
24 h ♂	Control	100	93	7	2	-	2	-	3	3.80
	125	100	87	13	3	-	1	-	9	3.20
	250	100	68	32	3	-	2	-	27***	3.00
	500	100	69	31	6	3	1	-	21***	2.80*

AP: Application Periods; Conc.: Concentration; OMN: Observed Metaphase Number; NMN: Normal Metaphase Number; NCA: Number of Cells with Aberrations; CG: Chromatid Gap; IG: Isochromatid Gap; CB: Chromatid Break; IB: Isochromatid Break; CA: Centromeric Attenuation; MI: Mitotic Index, *p<0.05, ***p<0.001

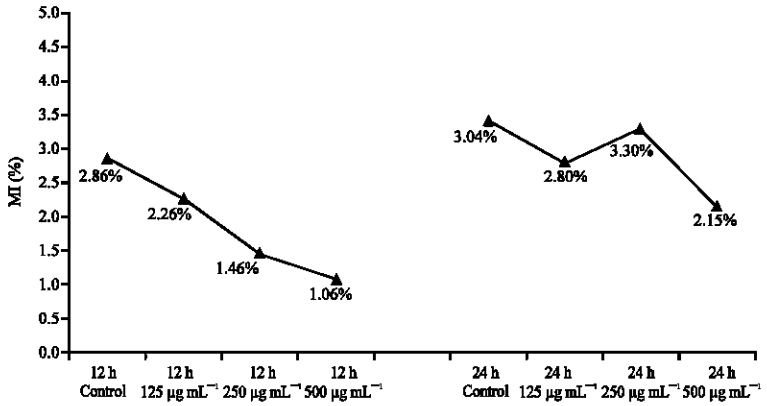

Fig. 3: MI values observed after Logran applications on female mice

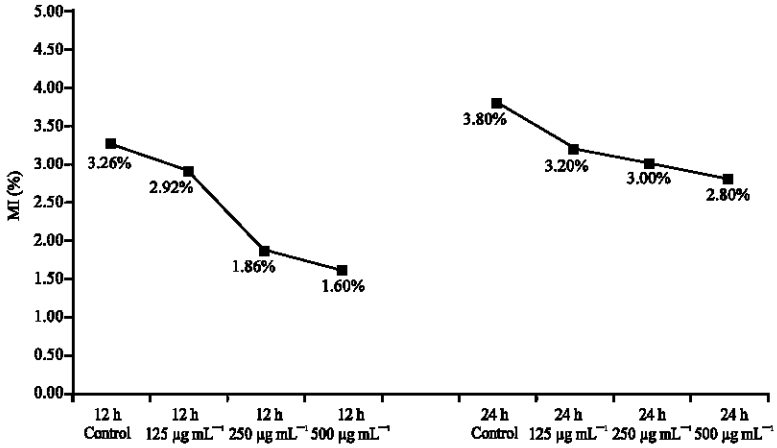

Fig. 4: MI values observed after Logran applications on male mice

Effect of Logran applications on mitotic index in both female and male mice were given in Fig. 3 and 4. A significant reduction for the mitotic activity ($p \leq 0.05$) was observed in both male and female mice for 12 h application period at 250 and 500 $\mu g\ mL^{-1}$ Logran. Mitotic index decreased with the increasing concentrations at this

period. For longer application period its toxicity was seen at the highest concentration (500 μg mL^{-1}). According to this data, 500 μg mL^{-1} Logran has cytotoxic effects in both application periods.

DISCUSSION

Some researchers have shown that pesticides induced chromosome aberrations in mice, rats and human lymphocytes (Blasiak and Stankowska, 2001; Jenssen and Renberg, 1976; Korte and Jalal, 1982; Lu *et al*., 2000; Madrigal-Bujaidar *et al*., 2001; Meng and Zhang, 1994; Papapaulou *et al*., 2001; Surralles *et al*., 1995). No such research has found about effect of Logran and its effective substance Triasulfuron on heredity material of *Mus musculus*.

Triasulfuron is one of sulphonylurea group herbicides. There were many conflicting reports about the genotoxicity of sulphonylurea compounds. Iodosulfuron-methyl-sodium which is a member of the sulphonylurea group herbicides did not induce chromosome aberrations and micronuclei in mammalian cells *in vitro* and bacterial assays (Marks, 2000). Linuron is one of the sulphonylurea compound. *In vivo* studies on Linuron genotoxicity in rats wasn't observed to increase in micronucleus frequency on bone marrow cells (Papapaulou *et al*., 2001). Ethametsulfuron-methyl was not genotoxic *in vivo* bone marrow chromosome aberration study in rats and micronucleus assay in mice (Gajanayake, 2002). In addition, some researchers have shown that positive effects of sulphonylurea herbicides on soil microbial biomass (El-Gramry *et al*., 2001), phytoplankton (Sabater *et al*., 2002) and plants (Kjaer and Heimbach, 2001). On the other hand Methylnitrosourea, Ethylnitrosourea produced chromosome aberrations in CHO cells and induced inhibition of DNA synthesis (Galloway *et al*., 1998). Linuron induced DNA damage in rat liver cells, Also Diuron induced the formation micronucleus on bone marrow cells of Swiss mice (Papapaulou *et al*., 2001). Metsulfuron-methyl was positive effective in CHO cells and induced chromosome aberrations (Dupont, 2001).

According to the results of these studies some sulphonylurea group herbicides: Linuron, Diuron, Metsulfuron-methyl have positive effects on mammalian cells. In the present study Logran which is a sulphonylurea herbicides induced chromosome aberrations on bone marrow cells of mice.

Logran has induced significant chromosomal aberrations in human lymphocyte culture (Muranlı and Kaymak, 2004). Kaymak and Muranlı (2006) showed that all the used concentrations of Logan significantly induced a number of choromosomal aberrations in root tip cells of *Hordeum vulgare* L. and *Triticum aestivum* L.

There are also some conflicting reports about genotoxicity of sulphonylurea group drugs used as hypoglycaemic agent. Chlorpropamide is one of these urea group drugs used as a hypoglycaemic agent in diabetic patients. Chlorpropamide significantly induced chromosome aberrations and chromosome exchange aberrations in the lymphocytes of these patients (Watson *et al*., 1976). In the study of Brown and Wu (1977) it is indicated that Chlorpropamide has increased number of SCE *in vitro* on Chinese hamster V79 cells. On the other hand in the study of Renner and Münzner (1980) it is indicated that Chlorpropamide and Tolbutamide have not induced chromatid aberrations and chromosome exchanges on Chinese hamsters and mice. Our results agree with these previous reports (Brown and Wu, 1977; Galloway *et al*., 1998; Watson *et al*., 1976).

In the present study centromeric attenuation was the most common abnormality that were observed on bone marrow cells of *Mus musculus* in both sexes. Many researchers observed centromeric attenuation as chromosomal aberration. In the study of El-Alfy (1998), centromeric attenuation displayed remarkable, progressive and significant increases on using the high dose of formalin on bone marrow cells of mice. Karima and Inas (1995) indicated that coadministration of diclofenac sodium and tolbutamide induced the highest frequency of centromeric attenuation. In the studies of Ali *et al*. (1998; 2001), carmoisine induced chromosomal aberrations including centromeric attenuation on the bone marrow cells and fluoxetine induced highly significant centromeric attenuation, respectively.

In the study of Van Hooser *et al*. (1998) mammalian centromere; structural domains and the attenuation of chromatin modeling is explained. It is indicated that there are centromer proteins CENPs-A, -B, -C (Brenner *et al*., 1981; Earnshaw and Rothfield, 1985), CENPs -G (He *et al*., 1998). The phosphorylation of histone H3 is essentially excluded from a specific region of centromeric chromatin by the presence of CENP-A (Van Hooser *et al*., 1998). The phosphorylation of histone NH_2-terminal tails has been proposed to reduce their affinity for DNA (Hendzel *et al*., 1997; Roth and Allis, 1992; Waring *et al*., 1997). In the absence of H3 phosphorylation and acetylation nucleosome structure is very tight and static. Phosphorylation, acetylation of histone tails is thought to weaken their association with DNA and open chromatin either directly by altering nucleosome structure (Norton *et al*., 1989) or indirectly by targeting nonhistone proteins (Turner, 1998).

Centromeric attenuation which was observed in the present study may be the result of some effected factors by the chemical, that has specific roles on chromatin modeling.

Cytotoxicity is defined as a decrease in mitotic index (Smaka-Kinci *et al*., 1996). The inhibition of mitotic activity in mice bone marrow cells reported in the present study indicates that Logran has a cytotoxic effect on these cells. It has been reported that many of the herbicides and insecticides inhibit mitotic activity (Lioi *et al*., 1998; Meng and Zhang, 1994; Papapaulou *et al*., 2001; Soloneski *et al*., 2002). Mitotic inhibition by herbicides has been attributed to blocking mitotic cycle which may result from prolonged G_2 period or to the inhibition of DNA synthesis (Chand and Roy, 1981). Also Schneiderman *et al*. (1971) have reported that reduction in the mitotic activity could be due to the inhibition of DNA synthesis. The decrease in mitotic index is correlated with chromosomal abnormalities. In this study, reduction of mitotic activity at 12 h application period was observed more than 24 h. This may be due to frequency of chromosomal abnormalities at 12 h application period.

In conclusion Logran significantly induced centromeric attenuation on bone marrow cells of *Mus musculus*. The cells were effected at 12 h application period more than 24 h application period. Moreover Logran reduced mitotic activity of the cells. As a result of this study Logran is genotoxic on bone marrow cells of *Mus musculus* according to the present test conditions. Other cytogenetic assays needed to be done to an exact conclusion of genotoxicity of Logran.

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