The Cytogenetic Effects of Logran on Human Lymphocyte Culture

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Summary In this study, the cytogenetic effects of a sulphonylurea group herbicide Logran (effective substance; Triasulfuron) were investigated in human peripheral blood lymphocyte culture. The cultures were treated with 2, 4, 8, 16, 32, 64, 128, and 256 $\mu\text{g/ml}$ concentrations of Logran for 24 and 48 h. This compound increased chromosome aberrations (CA) in human lymphocyte culture. Logran decreased Mitotic Index (MI) related to the concentrations. Between the concentrations of 16 and 256 $\mu\text{g/ml}$ Logran, this herbicide is cytotoxic and clastogenic in human peripheral blood lymphocyte culture treated *in vitro*.

Key words Logran, Herbicide, Chromosome aberrations, *in vitro* lymphocyte culture.

Nowadays humans are exposed to the effects of various chemical substances due to developing industry and technology.

These chemical substances can be agricultural pests, food additives and medicines. These compounds may have significant effects for many generations. Therefore, it is important to determine the mutagenic effects of these compounds by the appropriate test systems.

Sulphonylurea herbicides are used to control broad-leaved weeds. Logran is one of the sulphonylurea herbicides and its active substance is triasulfuron. This herbicide is used extensively, especially on wheat grown agricultural area in Turkish Trakya. It is known that about 10–12000 kg Logran is used on a 2–2.3 million hectare agricultural land in a single season in Edirne province. Excessive use of these sulphonylurea herbicides in agricultural areas may accumulate in soil and water. Thus, they may reach to the organisms and may result in several harms. Although the effects of some urea group herbicides are investigated (Agrawal *et al.* 1996, Chauhan *et al.* 1998, Papapoulou *et al.* 2001) there is no report about cytogenetic effects of Logran on animals and humans.

Testing agents for their ability to induce CA has a firm place in screening strategies for mutagenic/carcinogenic agents (Ishidate *et al.* 1998, Kirkland 1998, Obe *et al.* 1982). Detailed cytogenetic assay dealing with chromosome aberration is one of the important ways for evaluating genotoxic agents *in vivo* and *in vitro* (Carrano and Natarajan 1988, Natarajan and Obe 2002). CAs are induced by agents that damage chromosomal DNA (Natarajan 1976, Roberts 1978, Singer and Grunberger 1983). Therefore, scoring chromosomal aberrations is a direct measure of chromosome breakage and offers an accurate evaluation of the clastogenic (chromosome breakage) activity of an agent.

In the present study it was aimed to investigate the cytogenetics effects and dose response relations of Logran (commercial formulation of Triasulfuron) in human blood lymphocyte culture.

Material and method

In the present study, human peripheral blood was used as material, and Logran as a test substance. Logran consists of 4% triasulfuron ($\text{C}_{14}\text{H}_{16}\text{ClN}_5\text{O}_5\text{S}$ -3-(6-methoxy-4-methyl-1,3,5-triazine-

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2-yl)-1-(2-(2-chloroethoxy)-phenylsufonyl)urea), and 60% terbutyn ($C_{10}H_{19}N_5S$ 2 tert. butylamino-4-ethylamino-6-methylthio-s-triazine).

Concentrations of Logran were prepared by dissolving in distilled water. Two healthy and non-smoking females who were 23 and 26 years old were chosen as donors. 0.5 ml of blood taken from a donor was added to a 5 ml culture medium. Culture medium includes 10% Fetal Calf Serum (Sigma), 2% Phytoheamagglutinin (Sigma, CAS No. 9008-97-3), 100 IU/ml penicillin, 100 μ g/ml Streptomycin and Ham's F-10 (Sigma). These culture bottles were incubated for 72 h at 37°C. The cultures were treated with the final concentrations of 2, 4, 8, 16, 32, 64, 128, and 256 μ g/ml of Logran for 24 and 48 h. As for control group (negative control) the culture was not treated with the chemical substance but it was treated with distilled water. At 3 h prior to harvesting, 0.6 μ g/ml colchicine was added to arrest the cells at metaphase. After incubation, the cells were collected by centrifugation for 10 min at 1000 rpm. Then resuspended in a pre-warmed hypotonic solution (0.075 M KCl) for 25 min and fixed in glacial acetic acid: methanol (1:3 v/v). This fixation step was repeated until the lymphocytes' color were turned to white. Preparations were air dried and slides were stained with 10% Giemsa in Sørensen buffer for 10 min.

100 metaphases were scored for each concentration and treatment period and aberrations were recorded; chromatid gap, chromatid break, isochromatid gap, isochromatid break were determined. MI was determined by counting 2000 lymphocytes (MI was calculated as $MI = M/T$, where M represents the number of metaphase cells, T is the total number of cells scored).

In the present study, the results were tested with the Fishers' Exact χ^2 test both including (gap +) and excluding (gap -) gaps; the significance of the results of each treatment group was determined by comparing them with the control group.

Results

2, 4, 8 μ g/ml of Logran concentrations did not induce significant CAs excluding gaps (gap -) for both donors. If we accept gaps as CA (gap +) 8 μ g/ml concentration of Logran significantly induced abnormalities for 24 and 48 h treatment period in donor A and for 48 h treatment period in donor B. It was not needed to show the results of low concentrations of Logran (2, 4, 8 μ g/ml) in a table because of little changes in CAs.

Table 1 shows types and frequencies of structural chromosomal aberrations of higher concentration of Logran (16, 32, 64, 128 and 256 μ g/ml). According to the data a clear cut direct cytotoxic effect of Logran was seen in human lymphocytes. Logran decreased MI with the increasing concentrations. The highest concentration (256 μ g/ml) of Logran diminished mitosis in both donors except 24 h treatment in donor A. 128 μ g/ml concentration for 48 h treatment period also diminished mitosis in donor A, decreased MI to 0.5% in donor B. In donor A, after 24 h treatment period, 16, 32 and 256 μ g/ml concentrations of Logran, and after 48 h treatment period 16 μ g/ml concentration of Logran induced statistically significant CA (gap -).

In donor B after 24 h treatment period 32, 128 μ g/ml concentrations of Logran, and after 48 h treatment periods 64 and 128 μ g/ml concentrations of Logran induced statistically significant CA (gap -).

The herbicide Logran damaged the chromosome structure at concentrations between 16 and 256 μ g/ml, but no correlation was found between the concentrations and frequency of chromosome aberrations. This herbicide also induced CA for both 24 and 48 h treatment periods. Only in one donor (B) at 48 h treatment, more abnormal cells were observed compared to 24 h treatment period. 128 and 256 μ g/ml Logran concentrations after 24 and 48 h treatment period, diminished the amount of mitosis and brought about chromosomal changes difficult to estimate quantitatively. Therefore these concentrations could not be scored for CA induction shown as (-) in Table 1.

Our results show a dose dependent increase of abnormal cell number at all concentrations for

Table 1. Induction of chromosome structural aberrations in cultured human lymphocytes treated with Logran

Treatment Conc. ($\mu\text{g/ml}$)	Chromatid gap	Isochromatid gap	Chromatid break	Isochromatid break	Fragment	Total aberrations (-gap)	Total aberrations (+gap)	No. of cells with aberrations	% M.I.
I									
Control	1	0	1	0	0	1	2	1	9.20
Donor A	4	0	2	4	0	10**	14**	6	6.06
32	10**	2	4	1	2	8*	22***	7*	5.57
64	5	0	3	1	0	5	10*	4	3.65
128	3	1	1	0	1	2	7	2	3.24
256	11**	7**	0	2	2	6*	31***	4	1.80
I									
Control	3	0	0	0	1	1	4	1	9.04
Donor A	5	0	3	2	0	7*	12*	5	8.57
32	5	4	2	1	1	5	18**	4	8.15
64	4	3	2	0	0	2	12*	2	3.33
128	—	—	—	—	—	—	—	—	0.07
256	—	—	—	—	—	—	—	—	0.05
II									
Control	1	1	0	0	0	0	3	0	9.68
Donor B	2	2	0	0	0	0	6	0	8.10
32	5	0	2	1	1	5*	10*	4*	6.48
64	3	2	0	1	1	3	10*	2	4.96
128	5	0	0	4	1	9**	14**	5*	2.67
256	—	—	—	—	—	—	—	—	0.00
II									
Control	5	1	1	0	0	1	8	1	12.06
Donor B	8	2	1	0	0	1	13	1	8.39
32	18**	4	2	1	2	6	32***	5	7.25
64	18**	3	5	2	1	10**	34***	8*	4.08
128	26***	3	6*	2	1	11**	43***	9**	0.55
256	—	—	—	—	—	—	—	—	0.05

The total number of aberrations is calculated by assuming that one isochromatid break implies two breaks. 100 metaphases were scored for each concentration. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ (Fisher's exact test).

Fig. 1–4. 1. Observed aberrations after 128 $\mu\text{g/ml}$ Logran treatment for 48 h. a. Chromatid gap, b. Chromatid break ($\times 1000$). 2. Chromatid break observed after 64 $\mu\text{g/ml}$ Logran treatment for 48 h ($\times 1000$). 3. Chromatid break observed after 64 $\mu\text{g/ml}$ Logran treatment for 24 h ($\times 1000$). 4. Isochromatid break observed after 256 $\mu\text{g/ml}$ Logran treatment for 24 h ($\times 1000$).

two donors (gap +).

Some examples of CA are shown in Figs. 1–4.

Discussion

Chromosome aberration cytogenetic assay is one of the biological end points for evaluating genotoxic agents *in vivo* and *in vitro* (Carrano and Natarajan 1988, Natarajan and Obe 1982).

It has been shown by many investigators that pesticides induce CA in human lymphocytes (Cid and Matos 1987, Grant and Salamone 1994, Hrelia *et al.* 1996, Sinha *et al.* 1995). No such research exists about the effects of Logran and its compound Triasulfuron on hereditary material. However, in studies of Ciba-Geigy it is reported that this chemical regressed body development being toxic on liver and induced systemic toxicity in mice, rats, rabbits and dogs (Cid and Matos 1987, Ciba-Geigy 1985, 1986a, 1986d, 1988).

In the present study Logran induced CAs at concentrations between 16 and 256 $\mu\text{g/ml}$ in cultured human lymphocytes. Papapoulou *et al.* (2001) reported that Linuron (urea compound herbicide) induced micronuclei (MN) frequency and this induction resulted from breakage events as well as chromosome loss. Therefore, it is stated that Linuron is clastogenic in human lymphocytes. In the study of Agrawal *et al.* (1996) one of the urea herbicide Diuron induced the formation of MN and it was clastogenic in bone marrow cells of Swiss mice. Chlorpropamide (sulfonylurea drug) used as hypoglycemic agent significantly induced CA and chromosome exchange aberrations in lymphocytes of diabetic patients (Scasselati-Sforzolini *et al.* 1997). Although it has found that Chlorpropamide has not significantly induced chromatid aberrations and chromosome exchanges in chromosomal aberration test in Chinese hamster and mice (Renner and Münzner 1980), an increased number of sister chromatid exchanges (SCE) are found by investigations *in vitro* on Chinese hamster V79 cells treated with Chlorpropamide (Brown and Wu 1977). Watson *et al.* (1976)

reported that chlorpropamide acts on DNA molecule indirectly. Since it binds strongly to serum protein, it may produce its effects indirectly by affecting enzyme function.

In the present study Logran induced CA in cultured human lymphocytes without metabolic activation, although no correlation was found between the increase in frequency of aberrations and the herbicide concentration. Sheldon (1989) also found similar result with caprolactam without metabolic activation in human lymphocytes (Sheldon 1989). In the study of Walter *et al.* (1980) no correlation was found between the Malathion (insecticide) concentrations and frequency of CA (Walter *et al.* 1980).

Mitotic index (MI) data is an acceptable measure of cytotoxicity for lymphocyte cultures (Galloway *et al.* 1994, OECD 1997). In the present study Logran reduced mitotic activity of cells. Logran decreased MI with the increasing concentrations. For a variety of chemicals concentrations which give 50–70% suppression of MI can be extremely toxic concentrations (Armstrong *et al.* 1992). In the present study the concentration reduced MI of 50% up to 70% is 64 µg/ml. Lower concentrations (16 and 32 µg/ml) also reduced MI and induced CA. At all the concentrations it was seen a dose response reduction of mitotic activity. Logran is cytotoxic in human lymphocytes. It has been reported that many of the herbicides and insecticides inhibit mitotic activity (Lioi *et al.* 1998, Oraler *et al.* 1984, Surrales *et al.* 1995, Topaktas and Rencüzogulları 1994). Walter *et al.* (1980) indicated that Malathion (insecticide) is cytotoxic in human lymphocytes. Five pyrethroid insecticides, cypermethrin, deltamethrin, fenpropathrin, fenvalerate and permethrin, induced clear dose dependent cytotoxic effects (Surrales *et al.* 1995). Urea compound herbicide Linuron is cytotoxic in human lymphocytes (Agrawal *et al.* 1996) and in rat hepatic, bone marrow and testes cells (Scasse-lati-Sforzolini *et al.* 1997). Aldicarb is toxic in cultured human lymphocytes (Cid and Matos 1987).

In the present study the highest concentration (256 µg/ml) of Logran diminished mitosis for both donors except 24 h treatment in donor A. 128 µg/ml concentration for 48 h treatment also diminished mitosis in one donor and decreased MI to 0.05% in donor B. Walter *et al.* (1980) have found similar results with the insecticide Malathion with the concentrations 50 and 70 µg/ml for the same treatment periods in cultured human lymphocytes. Studies on the effects of cyneb and cyram on the mitotic activity of cultured cells revealed that higher concentrations of these pesticides diminish MI (Szichowa *et al.* 1977).

In conclusion, Logran induced significant CA in human lymphocyte culture although in the present test conditions no correlation was found between concentration and frequency of aberrations excluding gaps (gap –). Logran decreased MI related to the concentrations. Logran is cytotoxic and clastogenic in human lymphocyte culture without metabolic activation between the concentrations 16 and 256 µg/ml in the laboratory conditions. Therefore, further cytogenetic studies dealing with clastogenicity and genotoxicity of Logran may reveal more interesting results.

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