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THE CYTOGENETIC EFFECTS OF MALEIC HYDRAZIDE IN HUMAN LYMPHOCYTE CULTURE

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SUMMARY. In this study the cytogenetic effects of Maleic hydrazide (MH) at different concentration and treatment periods were investigated in peripheral blood culture. For this purpose, 75, 300, 500, 1000 µg/ml concentrations of MH dissolved in distilled water were used. Human lymphocytes were treated with MH at different concentrations for 24 and 48 hours. Chromosome abnormalities were observed such as chromatid gap, chromatid break, isochromatid gap and isochromatid break. Results were evaluated in two ways. If gaps are accepted as chromosome aberrations (CA), at all the groups MH significantly induced CA except 75 µg/ml 48 hour treatment group compared with control. If not, it was observed that MH is not affective. MH also decreased mitotic index (MI) depending upon the increasing concentrations and treatment periods.

Keywords: Maleic hydrazide, chromosome aberrations, human lymphocyte culture.

MALEİK HİDRAZİD'İN İNSAN LENFOSİT KÜLTÜRÜNDE SİTOGENETİK ETKİLERİ

ÖZET. Bu çalışmada Maleik Hidrazid'in (MH) farklı konsantrasyon ve muamele sürelerinde insan lenfositleri üzerindeki sitogenetik etkisi periferal kan kültürü yöntemiyle araştırılmıştır. Bu amaçla distile suda eritilen MH'in 75, 300, 500 ve 1000 µg/ml'lik konsantrasyonları kullanılmıştır. İnsan lenfositleri farklı konsantrasyonlardaki MH ile 24 ve 48 saat süre boyunca muamele edilmiştir. Kromatid gap, kromatid kırık, izokromatid gap ve isokromatid kırık gibi kromozom anormallikleri meydana geldiği saptandı. Sonuçlar iki şekilde değerlendirilmiştir. Gaplar kromozom aberasyonları (CA) olarak kabul edildiğinde 75 µg/ml MH'in 48 saat muamele edilmiş grup dışındaki tüm gruplarda MH insan lenfositlerinde kontrole göre anlamlı CA'larına sebep olduğu görülmüştür. Gaplar CA olarak kabul edilmediğinde MH'in insan lenfositleri üzerine etkili olmadığı saptanmıştır. MH artan konsantrasyon ve muamele süresine bağlı olarak mitotik indeksi (MI) de azaltmıştır.

Anahtar Kelimeler: Maleik hidrazid, kromozom aberasyonları, insan lenfosit kültürü.

INTRODUCTION

Every year number of pesticides increase which are used against agricultural pests. During the production, transformation, usage or after usage of them, these products accumulate in soil or water and therefore cause environmental pollution. Pesticides are contaminated to living organisms directly or with accumulation of them in food chains, and these cause not only to be poisinous but also they can be mutagenic and carcinogenic and they damage the genetic structure of living organisms.

Herbicides are the pesticides which are used mostly against agricultural pests. They are used to prevent growth of unwanted plants. These chemical substances which affect the plant metabolism on different periods, may harm the metabolism and kill the plants (Swietlinska &Zuk, 1978; Grover & Tyagi, 1979; Nicoloff et al., 1985; Gil & Navarrete, 1986; Patra et al., 1997; Xiao & Ichikawa, 1998) and genotoxic in animals (Cid & Matos, 1987; Grant & Salamone, 1994; Sinha et al., 1995).

The first time in 1951 it was reported that MH induced chromosome breaks (Patil & Bhat, 1992). MH that has been known as an effective chromosome breaking agent in higher plants, has been used as a depressant of plant growth (Swietlinska & Zuk, 1978). The chromosome breaking effect of MH on plant chromosomes resembles very closely the chromosome breaking properties of alkylating agents (Swietlinska & Zuk, 1978), and act in a similar way to that of alkylating agents, even though it is not an alkylating agent (Gil & Navarrete, 1986; Cortes et al., 1987).

However the effect of MH on bacteria and animals is not certain as in plants. While Swietlinska and Zuk (1978) has stated that MH is not toxic to bacteria and fungi, in 1986, Rashid and Mumma reported that MH induced DNA damage. Also different results can be obtained in the studies of mammalian cells with this chemical. While Perry and Evans (1975) have stated that MH didn't induce CA and sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells, Meschini et al. (1988) have reported that MH induced CA and SCE in CHO cells in the same conditions.

In the studies of MH in humans it has been indicated that MH inhibites the activities of human erytrocyte and plasma enzymes (Dowla et al., 1996; Panemangalore et al., 1999). Genotoxicity of MH in cultured human lymphocytes is studied by Ribas et al. (1995,1996). In the studies of Ribas et al. in 1995 it is stated that MH induced DNA breakage, in 1996 it induced SCE.

Since different effects of MH have been observed by different investigators, it is aimed to understand better the cytogenetic effects of this chemical in human blood lymphocyte culture.

MATERIAL AND METHODS

In the present study human peripheral blood is used as the material, and MH (Sigma, CAS no. 123-33-1) of which molecular formula is $C_4H_4N_2O_2$, as a test substance. The concentrations of MH was prepared by dissolving in distilled water in 50-60 ºC water bath. 1000 µg/ml concentration of MH is at the limit of solubility (Jewel, 1989). 1000 µg/ml and the half of this concentration 500 µg/ml and the concentrations that haven't been used before 75 µg/ml an 300 µg/ml were used in the present study.

One donor is used as in the studies of Topaktas & Spiet (1989)(1990), Cid & Matos (1984, 1987). 26 years old, healty, non-smoking female was chosen as a donor. Obtained lymphocytes by a standart macroculture technique were added to a 5 ml of culture into the bottle. Culture medium includes 10 % Fetal calf serum (Sigma), 2 % Phytohemagglutinin (Sigma, CAS No. 9008-97-3), 100 IU/ml penicillin, 100 µg/ml Streptomycin and TC 199 Medium (Sigma). These culture bottles incubated for 48 hours in 37 ºC. The cultures treated with the last concentration of 75 µg/ml, 300 µg/ml, 500 µg/ml and 1000 µg/ml of MH for 24 and 48 hours. The control culture have not been treated with a chemical. 4 hours prior to harvesting, 0,6 µg/ml colchicine was added to arrest the cells at metaphase. After incubation, the cells were collected by centrifugation 10 min. at 1000 rpm. Resuspended in a pre-warmed 37 ^{0}C hypotonic solution (0,075 M KCl) for 25 minutes and fixed in acetic acid:methanol (1/3 vol/vol). This fixation step was repeatedthree times. Air dried preparations were made and the slides were stained with 5 % Giemsa in Sörensen buffer for 10 min.

100 metaphases were scored for each concentration and treatment period, and the aberrations; chromatid gap, chromatid break, isochromatid gap, isochromatid break were determined. Experiment was repeated two times so metaphase number is shown as 200 in statistics. MI was determined by counting 2500 lymphocytes (MI was calculated as MI=M/T, where M represents the number of methaphase cells, T is the total number of cells scored).

The results were tested with the Fishers' exact X^2 test and the significancy of the results of each treatment group is determined by comparing them to the control group.

RESULTS

In this study, which is done by using human peripheral blood culture method, obtained results are given in table 1 releated to applied concentrations and periods.

For each group seen in 200 cells abnormal metaphase number were calculated in two ways with gaps (gap +) and without gaps (gaps -). If gaps are accepted as chromosome aberrations, MH significantly induced CA compared with control except 75 µg/ml 48 hours application group. If not it was observed that MH did not significantly increase CA at application groups compared with control.

Table 1. Observed results after treatment of MH at different concentration and treatment periods.
* $P \leq 0,05$ * * $P \leq 0,01$ * * * $P \leq 0,001$ (Fishers' Exact Test)

Concentration and treatment period	Observed metaphase number	Anormal Metaphase number (gap +)	Anormal metaphase number (gap -)	Chromatid gap	Iso-chromatid gap	Chromatid break	Iso-chromatid break	MI
Control	200	4	2	1	1	1	1	3,51
75 µg/ml 24 hour	200	13 *	6	5	2	5	1	3,25
300 µg/ml 24 hour	200	15 **	3	10 **	2	3	0	2,18
500 µg/ml 24 hour	200	18 **	9	6	3	8 *	1	1,73
1000µg/ml 24 hour	200	15 **	7	7	1	7	0	1,65
75 µg/ml 48 hour	200	10	5	3	2	4	1	1
300 µg/ml 48 hour	200	22 ***	7	13 **	2	7	0	0,80
500 µg/ml 48 hour	200	20 **	8	14 **	2	4	4	0,76
1000µg/ml 48 hour	200	15 **	8	6	1	8 *	0	0,40

The number of abnormal metaphase (gap +) was the highest for 24 hour application at 500 µg/ml concentration and for 48 hours application at 300 µg/ml concentration. Chromatid gaps were mostly seen at 48 hours 500 µg/ml concentration, chromatid breaks at 24 hours 500 µg/ml and 48 hours 1000 µg/ml concentrations. According to data CA number was higher at high concentrations. Some examples of CA are shown in figure 1,2. No significant difference was observed when abnormal metaphase number (gap-) of each group compared with control. Mostly seen abnormal metaphases were at 500 and 1000 µg/ml concentration groups. No difference was observed when application periods compared with each other.

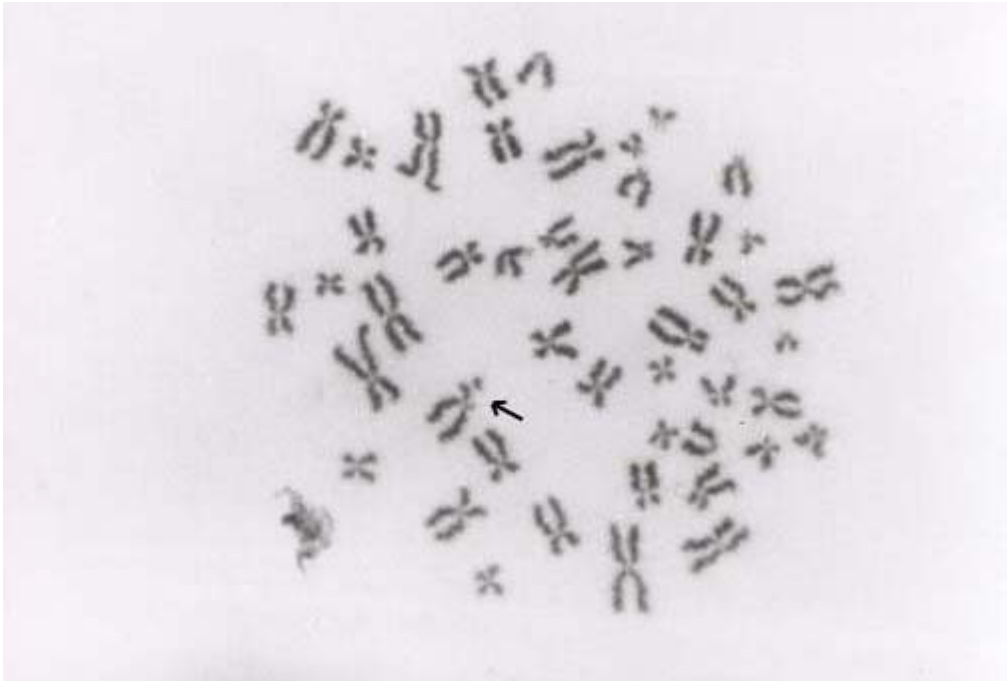

Figure 1. Observed aberrations after 500 µg/ml MH treatment for 24 hr. (a.isochromatid break, b. chromatid gap) (x1000).

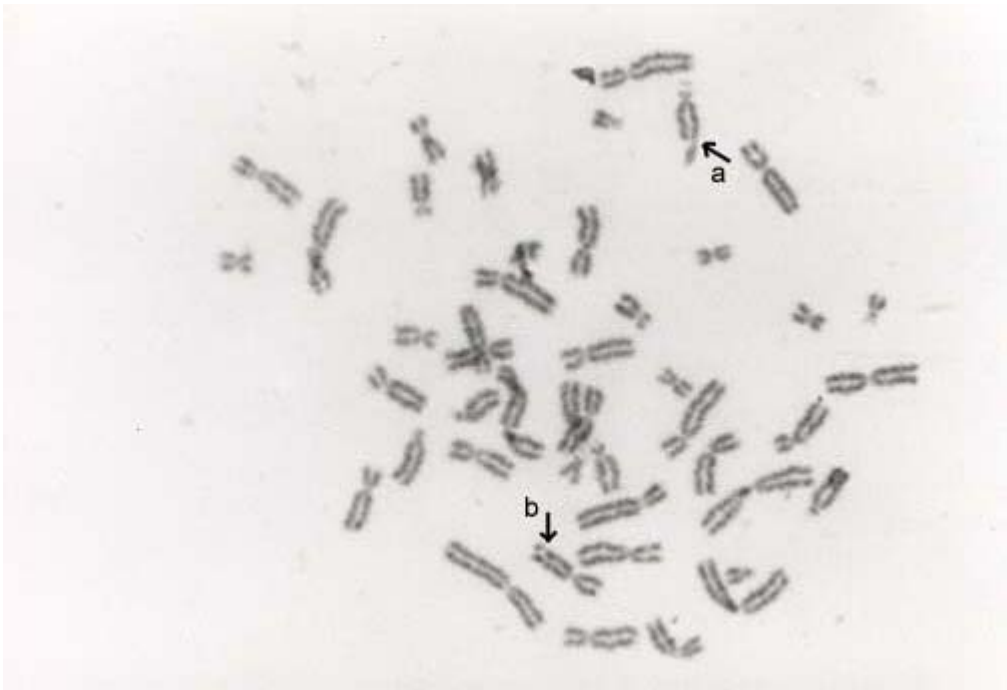

Figure 2. Chromatid break observed after 1000 µg/ml MH treatment for 48 hr. (x1000).

In our study, MI% of each application and control group was calculated. MH decreased MI releated to the concentration and application periods. Compared with control, at 1000 µg/ml concentration MH decreased MI from 3,51 to 0,4. The results obtained from treated and control groups are given in Figure 3.

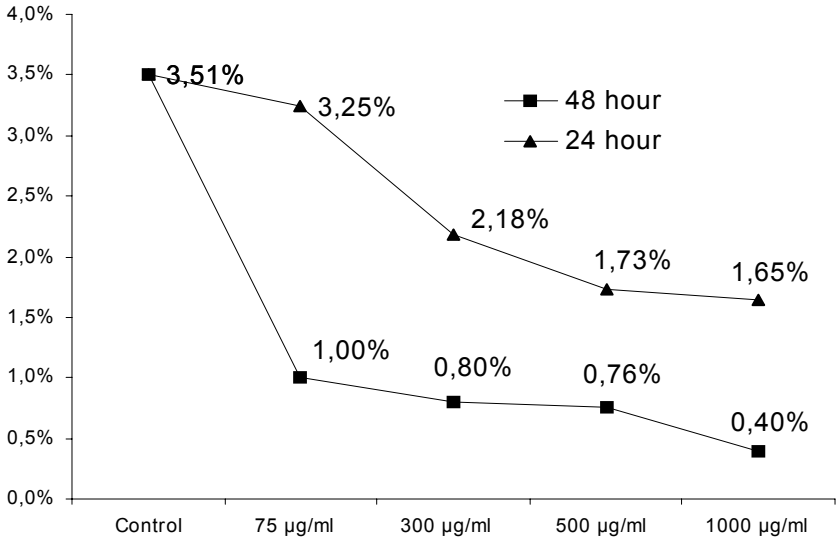

Figure 3. Observed MI value releated to the increasing concentrations and treatment periods.

DISCUSSION

Andersson (1993), studied spontaneous frequency of CA and sister chromatid exchanges (SCE) in human lymphocyte culture, and determined the aberration frequency between 0-4 per 100 cells. In our study, frequency of abnormality in control group was 1-2% consistent with findings in previous studies.

MH was used in this study is structural isomer of Uracil (Patil & Bhat, 1992), and a pyridazine that inhibits nucleic acid and protein synthesis (De Marco et al., 1992).

Following studies have shown that MH is an effective clastogen in plant cells inducing chromosome aberrations (Nicoloff et al., 1985; Andersson & Kıhlman, 1987; Mateos et al., 1989; Gil & Navarrete, 1986; De Marco et al., 1992; Kanaya et al., 1994; Rank & Nielsen, 1997; Gichner et al., 2000).

Swietlinska and Zuk (1978) have reported that although tests with some mammalian cell lines gave negative results MH induced CA in grasshoppers, fish and mice.

It is reported that an effective clastogenicity of MH in plant cells has suggested by many authors the existence of potent mutagenic metabolites after the bioactivation by the enzymatic system of plants (Ribas et al., 1996). In animals in the study of Takehisa & Kanaya in 1983 it has been shown that, after metabolic activation, MH induced significant increases in the frequency of SCE in CHO cells, suggesting that animal metabolism was also able to produce genotoxic metabolites in the biotransformation of MH (Ribas et al., 1996). It is reported that MH is also effective without metabolic activation. Meschini et al. (1988), have shown that MH induced SCE and CA in CHO cells without metabolic activation.

Ribas et al. (1995), have shown that MH induced DNA breaks in human lymphocyte culture with S9 fraction. In the study of the same researchers in 1996 it is shown that MH is not able to induce significant increase in CA. In the present study it is observed that without metabolic activation MH didn't induce significant increase in CA without gaps. This result is consistent with the findings in the studies of Ribas et al. in 1996.

It has been reported that many of the herbicides and insecticides inhibit mitotic activity (Cortes et al., 1987; Rao et al., 1988). Also it has been known that MH inhibits mitosis at higher concentrations in plants (Patil & Bhat, 1992). It has been found that MI decrease as a result of MH treatment in human lymphocytes (Ribas et al., 1996). In our investigation, we confirmed that MH decreased MI related to the concentrations and treatment periods.

According to the data in the present study, a significant increase of abnormal metaphase number (gap +), an increase of abnormal metaphase number (gap -) although not significant compared with control and a decrease in MI, suggest the thought that this chemical should be used carefully in agricultural areas.

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