Indirect Genotoxic Effect of Gamma Rays in Human Peripheral Lymphocytes

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Summary The aim of this study was to investigate the indirect genotoxic effect of various doses of gamma rays in human peripheral lymphocytes. For this aim, chromosome mediums were irradiated with various doses (2000, 4000, 8000, 16000 rad) of gamma rays.

In this study, we were found that SCE (Sister Chromatid Exchange) was increased by gamma rays doses-dependently. In addition to these, percentages of abnormal cells with chromosomal abnormalities and CA (Chromosome Aberration)/Cell were increased by all doses of gamma rays compared to control. Besides, gamma rays decreased the MI dose-dependently. RI was not also reduced at all concentrations.

Key words Human peripheral lymphocytes, Gamma ray, Indirect genotoxic effect, SCE, CA.

Problem of nourishing appeared together with increasing the population of the world. In the last 30 years, the sector of the storing the food products for a long time and increasing the food quality have become very important developments. The most important development of this sector is also irradiation technology. According to the experiments were made by researcher, ionising gamma rays have increased the endurance time of the foods, have decreased the number of the microorganisms of the foods, have destroyed the patojen microorganisms and parasites and also have impeded to be sprouted and to be vermin in herbal foods.

Firstly, X ray was used to against *Trichinella spiralis* by Schwartz (Schwartz 1921 from Uğur 1977). Mc Alister *et al.* (1991) found that gamma ray and ethylen oxide destroyed the natural microorganism populations that localized over the white and maize seeds. On the other hand, this method caused to change of the seed structure.

Hiemstra *et al.* (1991) observed that gamma radiation must be disregarded as a method for the sterilisation of plasma and plasma derived products, because of the fact that gamma rays didn't reduce the HIV virus activity.

The effective ingredients of chemical substance (Matteucci *et al.* 1993), the broths (Kornhof *et al.* 1994) and the solid poly polymers (ortho ester) that contaminated with *Bacillus bacterium* (Merkli *et al.* 1994) were sterilized by gamma rays but it was found that structure of these substances were damaged.

Shintani (1995) reported that methylenediailine that is a carcinogen was dose-dependent formed after gamma irradiation of poliürethan. Ten kinds of spice were sterilized with gamma rays at different doses (6, 12, 25 kGy) (Uğur 1997).

Although gamma rays were used to various sectors in order to sterilization, gamma rays caused to spoil and breakage of structure of substances that irradiated with high doses.

Mäder *et al.* (1996) investigated whether gamma rays caused to forming the free radicals after irradiated the polymers that used in medicine and was reported that the temperature of irradiation affect a little bit the forming of free radicals. However it was found that, the polymerase that was high melting temperature and cristallization was occurred max level of free radicals in room temperature after irradiation (Mäder *et al.* 1996).

It was founded that gamma and X rays directly induced chromosomal aberrations in human lymphocytes (Guedeney *et al.* 1988, Lefrançois *et al.* 1989, Lijima and Morimoto 1991, Milković-Kraus *et al.* 1992, Chambrette *et al.* 1996), in human fibroblast cells (Mühlmann-Diaz and Bedford 1995), in monkey lymphocytes (Guedeney *et al.* 1988, 1989, Van Buul 1983), in mouse spermatocytes (Van Buul *et al.* 1996) and in pollen mother cells of *Capsicum annuum* (Rao and Lakshmi 1980) and especially they increased heterozygote reciprocal translocation in barley pollen mother cells (Bilge *et al.* 1984). In addition, these rays also induced formation of micronuclei (Countryman and Heddle 1976, Huber *et al.* 1992, Köksal *et al.* 1996).

It was known that gamma rays caused to form free radicals in substances which irradiated sterilized. Foods and polymers which are used as a drug in medical treatment have been irradiated sterilized by gamma rays. As mentioned above, gamma rays have also directly damaged in genetic structure. For this reason, the aim of this study was to investigate whether induced the SCE and the CA in human peripheral lymphocytes incubated in Chromosome Medium B which is irradiated with various doses of gamma rays.

Material and methods

The 2.5 ml Chromosome Medium B (Biocrom F 5023) in steril tube were irradiated with 2000, 4000, 8000 and 16000 rad gamma rays (^{60}Co , Alcyon, 200 rad/min) within 30 min irradiation whole blood (0.2 ml) from 2 male and 1 female (age 23, 24, 26; non smokers) were added to 2.5 ml Chromosome Medium B irradiated with gamma rays. After this procedure, Bromodeoxyuridine (BrdUrd) (Sigma) ($10\text{ }\mu\text{g/ml}$) was added to medium and the cultures were incubated at 37°C for 72 h. And then Colchicine ($0.06\text{ }\mu\text{g/ml}$ final concentration) was added for the last 2 h of culture. To collect cells, the cultures were centrifuged (1200 rpm, 15 min), treated with hypotonic solution (0.4% KCl) for 33 min at 37°C , and then fixed in cold fixative (methanol: glacial acetic acid 3:1) for 20 min at room temperature. The treatment with fixative was repeated 3 times. The cells were spread on glass slides and air dried. The slides were stained by modified fluorescence plus Giemsa technique according to the method of Speit and Hauptner (1985) and mounted with entellan.

The significance between mean SCE, RI, MI and their controls were determined using the *t*-test. The significance between percentage of abnormal cell, CA/cell and their controls were determined using the χ^2 -test. Dose response relationship were determined from the correlation and regression coefficients and the corresponding regression lines.

Results

In this study, gamma rays indirectly induced the SCE. However, the mean of the SCE in human lymphocytes that incubated in medium irradiated with 16000 rad gamma rays were only increased compared to the control (Table 1). In addition gamma rays indirectly induced SCE dose-dependently (Fig. 1).

Table 1. The SCE, RI and MI in human lymphocytes that incubated in irradiated medium

Dosage (rad)	Min-Max SCE	SCE/Cell $\pm$ SE	M1	M2	M3	RI $\pm$ SE	MI $\pm$ SE
Control	1-12	4.47 ± 0.27	89	142	69	1.93 ± 0.06	1.93 ± 0.18
2000	2-13	4.99 ± 0.22	98	135	67	1.89 ± 0.07	$1.36\pm 0.12^*$
4000	1-14	4.86 ± 0.29	120	140	40	1.73 ± 0.12	1.46 ± 0.22
8000	1-16	5.16 ± 0.43	134	125	41	1.49 ± 0.21	$1.06\pm 0.19^*$
16000	2-13	$5.46\pm 0.20^*$	129	136	35	1.68 ± 0.05	$0.56\pm 0.15^{**}$

*: $P<0.05$, **: $P<0.02$.

At all doses, percentages of abnormal cells were significantly increased in human peripheral lymphocytes that incubated in irradiated medium compared to the control (Table 2). This increase was shown at a maximum rate in lymphocytes that incubated in medium that was irradiated by gamma rays at 4000 and 8000 rad.

The CA/cell was significantly increased compared to the control (Table 2). The maximum CA/cell was shown at 4000 and 8000 rad.

Gamma rays indirectly caused to various chromosomal aberrations like as sister union (Fig. 2), chromatid breakage (Fig. 3), chromatid exchange (Fig. 4). In addition gamma rays also caused to formed of gaps.

In this experiment, gamma rays indirectly decreased the MI compared to the control (Table 1). In addition this decreasing was dose-dependent (Fig. 5). However, gamma rays didn't also reduce the RI (Table 1).

Discussion

Indirectly induction of the SCE by gamma rays

In this study, gamma rays generally induced the SCE in human peripheral lymphocytes indirectly. But this increasing was found statistically significant compared to the control only at the cultures irradiated with 16000 rad gamma rays. In addition, gamma rays also increased the SCE dose-dependently.

It was found that the SCE was directly increased by irradiation after adding BrdUrd in mouse bone marrow cells (Morales-Ramirez *et al.* 1984). In addition it was reported that gamma rays directly increased the SCE in Bloom syndrome person who already have high level of the SCE (Aurias *et al.* 1985). It has been reported that 0.5 Gy gamma rays directly increased the SCE in CHO xrs-6c and K-1c cell lines irradiated at G₀ phase (Nagasawa *et al.* 1991). Moreover, it was reported that chlorophyllin prevented the SCE that were induced by gamma rays in murine bone marrow cells (Morales-Ramirez and Garcia-Rodriguez 1994). On the other hand, gamma rays didn't induce the SCE in human lymphocytes that irradiated with gamma rays at G₀ phase directly (Lijima and Morimoto 1991). In addition, Mühlmann-Diaz and Bedford (1995) also showed that both gamma rays and X rays didn't induce the SCE in human fibroblast which were irradiated with

Fig. 1. Correlation coefficient and regression lines of SCE in human lymphocytes that incubated in irradiated medium.

Table 2. The chromosomal abnormalities and gap, the percentage of abnormal cells and the CA/cell in human peripheral lymphocytes that incubated in irradiated medium

Dosage (rad)	Chromosomal Aberration and Gap*							Abnormal cell (%)	CA/Cell
	B'	SU	DS	T	CE	P	G		
Control	1	—	—	—	—	—	1	0.333±0.333	0.003±0.003
2000	—	8	1	—	—	—	2	3.000±1.155***	0.037±0.015****
4000	3	12	—	—	—	1	6	5.333±1.667****	0.057±0.019****
8000	—	12	1	—	1	—	—	4.667±1.333****	0.050±0.015****
16000	—	8	—	1	—	—	2	3.000±0.577****	0.037±0.009****

B': Chromatid Breakage, SU: Sister Union, DS: Disentric Chromosome, T: Translocation, CE: Chromatid Exchange, P: Polyploid Cell, G: Gap. *: P<0.05, **: P<0.02, ***: P<0.01, ****: P<0.001.

Figs. 2–4. 2) Sister union (8000 rad gamma ray, ♀). 3) Chromatid breakage (4000 rad gamma ray, ♂). 4) Chromatid exchange (8000 rad gamma ray, ♂).

Fig. 5. Correlation coefficient and regression lines of the MI in human lymphocytes that incubated irradiated medium.

1.5, 2.5, 4.5 and 7 Gy of gamma rays and X rays at G_0 and G_1 phase directly. As understood from our and other researcher results, SCE was induced directly and indirectly in some cell lines that gamma rays irradiated while in some cell lines the SCE weren't induced after irradiated with gamma rays. It was understood that the cell lines is very important for the investigating the genotoxic effects of rays using the SCE test system. In addition, feeding the cells also affected the SCE formation. On the other hand, cysteamine and chlorophyllin are radical scavenger properties. Reason of SCE which could not be increased in some cell lines may be arisen from the higher level of scavenger molecules (Özalpan 1980, Morales-Ramirez and Garcia Rodriguez 1994). As mentioned above, some researchers indicated that gamma rays were directly induced the SCE. Some researchers found that gamma rays didn't induce the SCE directly. On the other hand, Mendiola-Cruz and Morales-Ramirez (1989) also found that free radicals didn't induce the SCE. Whereas, we found that gamma rays indirectly induced the SCE in human peripheral lymphocytes. The difference between our and these researchers' results proceed from using different cell lines and irradiation doses.

Indirectly induction of the CA by gamma rays

In this study, gamma rays indirectly induced the CA at all doses. Chromatid breaks and sister unions were the most common abnormalities observed. Gamma rays directly induced the CA in human lymphocytes (Brandriff *et al.* 1988, Lefrançois *et al.* 1989, Lijima and Morimoto 1991, Milković-Kraus *et al.* 1992, Huber *et al.* 1992, Kosaka *et al.* 1995, Sing *et al.* 1995, Köksal *et al.*

1996, Chambrette *et al.* 1996, Garcia-Sagredo *et al.* 1996), in human chorionic villi (Salvi *et al.* 1993), in *Macaca mulatta* lymphocytes (Guedeney *et al.* 1988), in cynomolgus peripheral lymphocytes (Guedeney *et al.* 1989), in rhesus monkey (Van Buul 1989) and in mouse (Sjoblom *et al.* 1995, Zang 1995). It is shown that gamma rays directly and indirectly affected the DNA and caused to chromosome mutation.

In this study, it was not observed that any dose-response relationship. Because the CA at 16000 rad is less than 4000 and 8000 rad. This situation may be caused from eliminating of abnormal cells at 16000 rad. In this study, SU has been observed as the most abnormality due to the terminal deletion. Sasaki and Matsubara (1977) reported that the chromatid exchange which was caused from free radicals wasn't repaired while the terminal deletion was repaired. In this study, it was shown that the terminal deletion was not repaired because of forming of the SU as the most common abnormality. In addition the doses that used in this study (2000, 4000, 8000, 16000 rad) were rather higher than the doses that used by Sasaki and Matsubara (1977) (300 rad).

Indirectly cytotoxic effect of gamma rays

In this study, MI was decreased dose-dependently but RI was not affected. For this reason, it was understood that the gamma rays decreased the MI *via* the affecting of G₂ and prophase. However, the decreasing of the MI might be caused by the decreasing of the ATP level and the pressure from the functioning of the energy producing centre (Epel 1963, Jain and Andsorbhoy 1988).

As a result, free radicals have formed in chromosome medium which was irradiated by gamma rays and it was also known that free radicals have genotoxic effect. We are opinion that free radicals should be measured in food that sterilized by irradiation and should be also determined the time-dependently decreasing of the quantitive of the free radicals.

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