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Variability of chromosomal radioadaptive response in human lymphocytes

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ABSTRACT

Background: There are growing evidences for chromosomal radioadaptive response in human lymphocytes. Highly variable inter- and intra-individual responses have been reported. Some individuals are non-responders and even in some donors the frequency of chromatid aberrations induced by a challenge dose increases by pre-exposure to an adapting dose. It has been proposed that the lack of radioadaptive response is due to transient physiological factors.
Materials and Methods: We found a young healthy donor who exhibited no radioadaptive response in our initial experiments. After a common adapting dose, the donor occasionally showed a highly increased susceptibility to subsequent high-dose irradiation. To assess whether the lack of radioadaptive response and the induction of a synergistic effect are transient responses, we have performed a 3-year follow-up study employing micronuclei in binucleated cells besides chromatid aberrations as biological endpoints. To eliminate the effect of the cell cycle on intrinsic radiosensitivity of a cell, we used the multiple-fixation regimen for analysis of chromosomal aberrations.
Results: This donor showed no adaptive response in any experiment.
Conclusion: Considering the consistent non-responsiveness observed throughout our serial experiments, it may be concluded that the lack of radioadaptive response is not attributed to some transient physiological factors but rather to permanent constitutional traits. *Iran. J. Radiat. Res., 2003; 1(1): 55 - 61.*

***Keywords**: Radioadaptive response, chromosomal aberrations, human lymphocytes, synergistic effect, CB-micronucleus assay.*

INTRODUCTION

When living organisms are exposed to a variety of DNA damaging stresses such as UV, alkylating or oxidizing agents and heat, adaptive responses are induced which render them resistant to the killing and mutagenic insults (Samson and Cairns 1977). This type of reduced radiation susceptibility after exposure to ionizing radiation was first reported by Olivieri *et al*. (1984). Cultured human lymphocytes exposed to a low dose of ionizing radiation had fewer chromatid aberrations induced by a subsequent high dose, compared to lymphocytes not pre-exposed to a low dose. It has been recently reported that above the normal levels of natural radiation can induce adaptive responses in human lymphocytes. Cultured lymphocytes of the residents of the areas with high levels of natural radiation in Ramsar, Iran, when exposed to a 1.5 Gy dose of gamma radiation, showed fewer chromatid aberrations

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than those of the control group (Ghiassi-Nejad *et al*. 2001). Based on the results obtained from the studies on high background radiation areas, it has been suggested that radioadaptive response may have implications in radiation risk assessment (Mortazavi 2002) and radiation protection (Karam *et al*. 2002). These studies also have opened new horizons in radiation protection against high levels of cosmic radiation during long-term space travel (Mortazavi *et al*. in press).

On the contrary, the absence of radioadaptive response has been long reported in cultured human lymphocytes. There is an inter-individual variability with respect to the induction of radioadaptive response (Sankaranarayanan *et al*. 1989, Bosi and Olivieri, 1989, Bauchinger *et al*. 1989, Hain *et al*. 1992, Vijayalaxmi *et al*. 1995, Kalina and Nemethova, 1997, Gadhia, 1998). In some cases, the existence or lack of adaptive response in a donor varied with time (Olivieri and Bosi 1990). It has been proposed that some transient physiological factors might contribute to the variability of radioadaptive response. On the other hand, inter-individual variability was not considerable in monozygotic twins, while dizygotic twins showed greater variability; this indicates an important role of genetic constitution as a source of variability (Kalina and Nemethova, 1997). In late 1998, we found a young healthy donor who did not exhibit radioadaptive response in any experiment and occasionally showed synergistic response after exposure to a common adapting dose (Ikushima and Mortazavi 2000). In the present study, we have tested whether the lack of adaptive response and the induction of this synergistic effect are permanent phenomena. To eliminate the effect of the cell cycle on intrinsic radiosensitivity of a cell, we used the multiple- fixation regimen for the analysis of chromatid aberrations and also employed micronuclei in binucleated cells as the second endpoint.

MATERIALS AND METHODS

Whole blood culture

The selected donor for this long-term follow-up study was a healthy male non-smoker aged 34. Peripheral blood was drawn from this donor and other healthy donors into heparinized vacutainers. Separate cultures were set up from each blood sample, using 1 ml blood in 9 ml RPMI 1640 medium (with 25 mM Hepes buffer and L-glutamine), containing 20% fetal bovine serum (Bio Whittaker) and 2.5% phytohemagglutinin (PHA, Gibco BRL). The cultures were incubated in dark, at 37^{o} C in a humidified atmosphere of 95% air and 5% CO_2.

X-ray irradiation

The cells were exposed to the adapting dose of 5 or 10 cGy X-ray (SOFTEX X-ray machine model M-150WS, 70-150 kVp, 5 mA, 0.1 mm Cu + 0.5 mm Al filter, dose rate 0.247 or 0.099 Gy/min) at 24 h after PHA stimulation and/or to the challenge dose of 2 or 3 Gy X-ray (the same irradiation factors) at 48 h. After the challenge dose, the culture flasks were returned to the incubator for a further incubation of 6 h.

Chromosomal aberration analysis

Colcemid (Gibco BRL) was added 2 h before harvesting at a final concentration of 0.25 µg/ml to arrest the dividing lymphocytes at metaphase. In the multiple-fixation regimen, colcemid was added 50, 52 and 54 h after stimulation. After harvesting, the cells were treated with 0.075 M KCl for 10 min at 37^{o}C and fixed with methanol-acetic acid (3:1 *v/v*). The fixed cells were dropped onto wet slides, air dried and stained with 2% Giemsa (Merck) in 1/15 M phosphate buffer at pH 6.8 for 15 min. For each data point, 100 - 400 well-spread metaphases with 46 chromosomes were examined for chromosomal aberrations. The scoring was restricted to chromatid and isochromatid breaks as well as chromatid exchanges. Achromatic lesions or gaps were not included in data analysis.

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Cytokinesis-block micronucleus assay

Cytochalasin-B (Sigma) was added to the cultures at 48 h after PHA stimulation at a final concentration of 4μg / ml to block cytokinesis of the dividing lymphocytes. After an incubation period of 72 h, the cells were collected by centrifugation and treated with a cold 0.075 M KCl hypotonic solution. To preserve the cytoplasm, centrifugation was done immediately after the addition of the KCl. Then the cells were fixed in freshly prepared cold fixative (10:1:11 methanol: acetic acid: Ringer's fluid). Following the first fixation, the cells were washed twice with freshly prepared methanol: acetic acid (6:1 *v/v*). After centrifugation and discarding the supernatant, cell suspensions were carefully dropped onto wet slides. Slides were air dried and stained with 4% Giemsa (Merck) in distilled water for 10 min and then mounted. At least 1000 binucleate lymphocytes with preserved cytoplasm were scored blind for each data point. Criteria used for scoring the micronuclei were those described by Fenech (1993). The diameters of the micronuclei were less than one-third of the main nuclei.

Data analysis

The expected frequency of chromatid aberrations or micronuclei was calculated as follows:

Observed frequency=frequency in cells exposed to a challenge dose after treatment with a low adapting dose

Expected frequency=frequency of adapting dose alone + frequency of challenge dose alone – frequency of control

In this equation, frequency of adapting dose alone is the frequency obtained in the cells only exposed to an adapting dose. Frequency of challenge dose alone is the frequency in the cells only exposed to a challenge dose, while frequency of controls is the frequency in cells exposed neither to an adapting dose nor to a challenge dose.

Furthermore, the coefficient of induced adaptive response (k) was calculated as the ratio of the observed frequency to the expected frequency.

Standard error of the k-value (SE_k) was calculated according to the formula:

$$(SE_k/k)^2 = (\text{SE observed}/\text{Observed frequency})^2 + (\text{SE expected}/\text{Expected frequency})^2$$

In this formula, SE observed and SE expected are standard errors of observed and expected frequency, respectively. When the k value is less than 1, it indicates that a positive radioadaptive response occurred. If k=1, it means a simple additivity effect. When k exceeds 1 significantly, it means that a synergistic effect was induced.

The statistical differences between observed and expected values for chromatid aberrations and micronuclei were determined with Student's *t-test*.

RESULTS

The frequencies of chromatid aberrations in the initial experiment are summarized in table 1. They were obtained in lymphocytes from 4 healthy donors under the standard schedule of adapting and challenging exposures. Interestingly, the first donor showed a significant synergistic effect ($p < 0.01$) while all of the three other donors exhibited a significant radioadaptive response. The k-value of the responders ranged from 0.74 to 0.79, which indicates a considerable variability in the magnitude of the induced radioadaptive response between individuals. For the non-responder donor, two types of experiments were repeatedly performed at 6-month interval, employing different adapting and challenge doses.

Table 1. Frequency of chromatid aberrations in human lymphocytes treated with 5 cGy followed by 2 Gy of 150 kVp X-rays.

	No. of chromatid aberrations per cell[a]			
Donor	1st	2nd	3rd	4th
Observed frequency	0.60	0.45	0.46	0.62
Expected frequency	0.32	0.60	0.62	0.78
p-value	< 0.01	< 0.05	< 0.05	< 0.05
Response	Synergistic	Adaptive	Adaptive	Adaptive
k-value	1.86	0.75	0.74	0.79

400 cells were scored for each point.

[a] 0.02 or less in non-irradiated cells and 0.05 or less after exposure to a 5 cGy adapting dose.

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Table 2 shows the frequency of chromatid aberrations induced by challenge dose of 3 Gy in the cells pre-exposed to adapting dose of 10 cGy, using X-rays with different maximum energies (70-150 keV). A significant synergistic effect was observed in 3 different voltages ($p < 0.01$, P < 0.01 and $p < 0.05$ for 70 kVp, 130 kVp and 150 kVp respectively) except 100 kVp. In these experiments, the k-values ranged from 1 (no response to adapting dose) to 1.66, which indicates a considerable magnitude for the induced synergistic effect. The overall results of two experiments in the cells pre-exposed to 5 cGy and challenged by 2 Gy are shown in table 3. Despite the existence of synergistic effect only for X-rays produced with one voltage ($p < 0.05$ for 130 kVp X-rays), radioadaptive response has never been observed in any voltages. The k-values in this experiment ranged from 0.92 to 1.31, which indicates a more limited range for the coefficients of the induced response.

Table 2. Frequency of chromatid aberrations in lymphocytes of the non-responder donor exposed to 10 cGy followed by 3 Gy of X-rays with different maximum energies.

	No. of chromatid aberrations per cell[a]			
X-ray tube voltage (kVp)	70	100	130	150
Observed frequency	0.53	0.46	0.61	0.68
Expected frequency	0.36	0.46	0.39	0.50
p-value	< 0.01	NS	< 0.01	< 0.05
Response	Synergistic	None	Synergistic	Synergistic
k-value	1.47	0.78	1.59	1.36

200 cells were scored for each point.

[a] 0.02 or less in non-irradiated cells and 0.04 or less after exposure to a 10 cGy adapting dose. NS: not significant.

We re-assessed the radioadaptive response in the non-responder donor three years after the initial experiment. Table 4 shows the frequencies of the chromatid aberrations in the non-responder and three control donors. Interestingly, the non-responder showed no radioadaptive response again. The observed frequency of chromosomal aberrations is still higher than the expected value, indicating a synergistic effect. However, this synergistic effect was not statistically significant. One of the control donors showed a significant radioadaptive response ($p < 0.05$), while the induced radioadaptive response in two other controls were not statistically significant. The k-value in the non-responder is 1.33, while it ranged from 0.5 to 0.86 in controls.

Table 3. Frequency of chromatid aberrations in lymphocytes of the non-responder donor exposed to 5 cGy followed by 2 Gy of X-rays with different maximum energies.

	No. of chromatid aberrations per cell[a]			
X-ray tube voltage (kVp)	70	100	130	150
Observed frequency	0.60	0.45	0.46	0.59
Expected frequency	0. 50	0.36	0.35	0.64
p-value	NS	NS	< 0.05	NS
Response	None	None	Synergistic	None
k-value	1.20	0.25	1.31	0.92

200 cells were scored for each point.

[a] 0.01 or less in non-irradiated cells and 0.03 or less after exposure to a 5 cGy adapting dose . NS: not significant.

Table 4. Frequency of chromatid aberrations in lymphocytes of the non-responder and 3 new control donors treated with 5 cGy followed by 2 Gy of 100 kVp X-rays.

	No. of chromatid aberrations per cell[a]			
Donor	Non-responder	1st	2nd	3rd
Observed frequency	0.20	0.15	0.08	0.12
Expected frequency	0.15	0.18	0.16	0.14
p-value	NS	NS	< 0.05	NS
Response	None	None	Adaptive	None
k-value	1.33	0.83	0.50	0.86

200 cells were scored for each point.

[a] 0.02 or less in non-irradiated cells and 0.05 or less after exposure to a 5 cGy adapting dose. NS: not significant.

Using the multiple-fixation regimen to

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eliminate the effect of cell cycle on the intrinsic radiosensitivity of cells, we determined the frequency of chromatid aberrations in the non-responder donor's cells fixed at three successive times (table 5). Despite a variety in the frequency of chromatid aberrations, no adaptive response was observed again at any fixation times. The k-values ranged from 1.21 to 1.43 indicated the induction of a synergistic effect. However, the induced synergistic effect was statistically significant only for the cells fixed at 52 h.

Table 5. Frequency of chromatid aberrations in lymphocytes of the non-responder donor fixed at successive times after treatment with 5 cGy followed by 2 Gy of 100 kVp X-rays.

	No. of chromatid aberrations per cell[a]		
Fixation time after challenging dose (h)	**4**	**6**	**8**
Observed frequency	0.93	0.20	0.58
Expected frequency	0.65	0.14	0.48
p-value	< 0.05	NS	NS
Response	Synergistic	None	None
k-value	1.41	1.43	1.21

200 cells were scored for each point.

[a] 0.01 or less in non-irradiated cells and 0.04 or less after exposure to a 5 cGy adapting dose. NS: not significant.

As shown in table 6, even when the cytokinesis-block micronucleus technique was used as an another test system, the non-responder exhibited no radioadaptive response but a significant synergistic effect (p<0.001) again. Among the three new control donors, two individuals showed a significant radioadaptive response. One of them has also shown a significant radioadaptive response for chromatid aberrations (table 4), indicating that the simplicity of the cytokinesis-block micronucleus assay should ensure its application in epidemiological survey of radioadaptive response within a large number of people. The k-value for the non-responder was 1.2 while it ranged from 0.58 to 1.07 for controls. It should be noted that the radioadaptive response negative donor has never turned positive throughout the present study.

Overall results of our serial experiments indicate the existence of a significant radioadaptive response in 5 out of 9 healthy donors.

Table 6. Frequency of micronuclei in binuclei cells of the non-responder and 3 control donors exposed to 5 cGy followed by 2 Gy of 100 kVp X-rays.

	No. of chromatid aberrations per cell[a]			
Donor	Non-responder	1st[b]	2nd	3rd
Observed frequency	0.30	0.24	0.31	0.23
Expected frequency	0.24	0.40	0.31	0.39
p-value	< 0.001	< 0.001	NS	< 0.001
Response	Synergistic	Adaptive	None	Adaptive
k-value	1.25	0.58	1.0	0.59

1000 cells were scored for each point.

[a] 0.02 or less in non-irradiated cells and 0.05 or less after exposure to a 5 cGy adapting dose.
[b] The 1st donor is the 2nd donor in table 4. NS: not significant.

DISCUSSION

It has been reported that the lack of radioadaptive response in some donors is not linked to their genetic constitution but it depends on some transient physiological factors. Our three-year follow-up study on a non-responder clearly showed that the lack of radioadaptive response is not a transient phenomenon but rather it depends on some non-transient factors such as genetic constitution of each individual. To date the cause of the lack of radioadaptive response in some individuals is not clearly known. Furthermore, the origin of the induced synergistic effect is still an open question. During the last decade some investigators have investigated the possible causes of absence of radioadaptive response or synergistic effects. The possible causes are mostly attributed to the following factors:

Transient physiological parameters

Some of the investigators have proposed that

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the existence or lack of radioadaptive response depend on transient physiological parameters. Olivieri and Bosi (1990) indicated that the failure to show a radioadaptive response is a consequence of the physiological state of the cells at the time of low-dose irradiation. They found that repeating the experiments with specified donors who did not show radioadaptive response previously, altered both the negative results into positive and positive results into negative. They concluded that the variability of the radioadaptive response is not linked to genetic constitution of the individuals but depends on some transient physiological parameters.

Genetic constitution of individuals

It has been proposed that genetic constitution of each individual determine the presence or absence of radioadaptive response. Kalina and Nemethova (1997) showed that individual differences in radioadaptive response between the monozygotic twins were negligible but in the case of dizygotic twins, these variations were much greater and were comparable to those observed in unrelated individuals. Our results are consistent with this proposal. Indeed, our experiments indicate that it is probably impossible for our radioadaptive response a negative donor turns to positive.

Genetic disease and chromosomal abnormalities

It is now known that the probability of being a non-responder among patients with chromosome instability syndromes is higher than among healthy persons. Khandogina *et al*. (1991) observed that 5 donors out of 6 patients with Down's syndrome did not show radioadaptive response. Nemethova *et al*. (1995) also found no radioadaptive response in ataxia telangiectesia homozygotes either after a low dose of gamma rays or after a low dose of bleomycin. Obviously, we can not use these findings for explaining the origin of the absence of radioadaptive response in our non-responder. The low frequency of chromosomal aberrations in non-irradiated lymphocytes as well as cells irradiated only with adapting dose excludes the possibility of any chromosomal instability in our non-responder. This view is supported by the observation that the frequency of chromatid aberrations induced by challenge dose alone is rather lower in our non-responder than in other control donors.

Aging

Gadhia (1998) recently reported that aging could be a factor, which abolishes the adaptive response. The existence of radioadaptive response in the blood of all donors aged 5-45 years and absence of radioadaptive response in all of 12 donors aged 65 suggests that possibly radioadaptive response is age dependent. In contrast with Gadhia's results, our non-responder, aged 34 years, always showed the lack of any statistically significant radioadaptive response in all of our numerous experiments using different end-points, multiple fixation times and different radiation qualities, indicating that the absence of radioadaptive response is not caused by aging.

These findings suggest that radioadaptive response does not necessarily depend on transient physiological factors but possibly on the genetic constitution of individuals. Since the knowledge of the origin of absence of radioadaptive response is very important and helpful in elucidation of the mechanisms of adaptive response, we recommend that similar long-term follow-up studies should be performed with a relatively large number of adaptive response negative donors. Despite the fact that we do not know the frequency of such individuals who show a synergistic effect in the population, it may be concluded that possible implications of radioadaptive response in the estimation of the risks of low-level radiation exposure are still problematical.

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