Chromosome Aberrations in Cultured Peripheral Lymphocytes from Persons with Elevated Skin Radiosensitivity

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The purpose of this study was to elucidate whether an enhanced skin radiation reaction correlated with an enhanced chromosome radiation response. Twelve patients with late radiation skin ulcers formed after courses of radiation therapy were chosen as a group of individuals with elevated skin radiosensitivity. Half of the venous blood samples from each donor were irradiated with 2 Gy γ-rays; the other half remained unexposed. Using standard cytogenetic technique, lymphocyte cultures were prepared with all samples. On the metaphase preparations, all chromosome aberrations detectable without karyotype identification were scored. The frequency of various aberrations in each patient were compared with relevant mean values in healthy unexposed donors. In several patients, the frequency of one aberration type or another exceeded the control value significantly. Comparison of aberration patterns in irradiated and unirradiated cultures and consideration of elapsed time after therapeutic exposures suggested that the observed increased aberration levels reflected individual features of the patients' radiosensitivity, rather than lesions induced by previous in vivo exposures. Therefore, the question of a correlation between skin and chromosome radiosensitivity can be answered positively. Analysis of the peculiarities of cellular distribution of aberrations and of the relative contribution of different aberration types in patients and healthy donors indicates that the investigation of in vitro-induced aberrations is more suitable for the assessment of individual radiosensitivity than the study of aberrations observed in unexposed cultures. — Environ Health Perspect 105(Suppl 6):1437–1439 (1997)

Key words: late radiation injuries, peripheral lymphocytes, chromosome aberrations, human radiosensitivity

Introduction

In approximately 20% of cases, standard therapeutic exposures result in formation of late radiation skin ulcers (1). Treatment of late radiation injuries is very complicated, if not impossible. One plausible reason for ulcer formation lies in an elevated individual skin radiosensitivity, and it would be very useful to be able to select such sensitive individuals before the onset of radiation therapy. In the search for criteria for such a selection, we tried to determine if individual skin radiosensitivity correlates with individual chromosome radiosensitivity measured by chromosome reaction to in vitro irradiation. In this study, we used peripheral lymphocytes, as they have been thoroughly investigated in radiobiology and cytogenetics, and because blood radiosensitivity, though by no means a perfect reflection of the general organism radiosensitivity, is one of its essential characteristics. To date, we have examined 12 patients in the clinic of the Medical Radiology Research Center (Obninsk) with late radiation skin injuries. These patients are regarded as persons with possibly elevated individual skin radiosensitivity. The response of their chromosomes to in vitro irradiation has been compared with the response in healthy individuals.

Methods

Venous blood samples were taken from 12 patients who experienced standard local therapeutic exposures and from seven healthy unexposed volunteers. Total exposure doses were approximately 70 Gy; time periods after exposures are given in the next section. One-milliliter aliquots of whole heparinized blood were exposed in culture flasks to 2 Gy of 60Co γ-rays at 20 rad/min. Nine milliliters of minimal essential medium containing glutamine, bovine serum (15%), penicillin, and phytohemagglutinin was added immediately to both irradiated and nonirradiated blood samples. After 51 hr incubation at 37°C (with colchicine added for the final 3 hr) the cells were treated with hypotonic KCl, fixed with ethanol/acid (3/1) mixture, spread onto slides, and stained with azur-eosine.

Metaphases with 45 to 47 chromosomes were analyzed and all aberrations detectable without karyotype identification were scored. Dicentrics, centric rings, detectable symmetrical translocations, double acentric rings (including minutes), and free double fragments were registered as chromosome-type aberrations, whereas all kinds of intrachromatid exchanges, single fragments, and isochromatid aberrations were ascribed to the chromatid type.

Results and Discussion

In healthy donors the results obtained for each type of aberration were homogenous both in unexposed and irradiated cultures (p > 0.9, chi-square test), so they were pooled, and the mean values were taken as control. These values are in accordance with published data (2,3). The values for the patients were not homogenous (p < 0.02), so the comparison of aberration frequencies with the control values was made separately (by t-test) for each patient. The results of this comparison are summarized in Table 1, which also shows the time period between the end of therapeutic exposure and the date when each patient's blood sample was taken for this study. The data marked with asterisks in Table 1 correspond to an increased frequency of various types of chromosome damage in some patients above the control level.

Table 1 shows that in 6 of 12 patients (patients 3, 4, 5, 6, 8, 12), frequency of one type of chromosome damage or another was elevated significantly (p < 0.05), whereas the rest of the patients did not
Table 1. Frequencies of different types of chromosome damage in cultured lymphocytes from patients with late radiation skin injuries.

| Patient | No in vitro exposure | 2 Gy in vitro |
|---------|----------------------|---------------|
| No | Time, yearsa | AM, % | CsE/100 cells | CsE/100 cells | AM/100 cells | CsE/100 cells | CsE/100 cells | AM/100 cells | CsE/100 cells | CsE/100 cells |
| Control | – | 1.88 ± 0.48 | 0.50 ± 0.25 | 0.13 ± 0.13 | 1.38 ± 0.41 | 42.26 ± 1.74 | 18.09 ± 1.50 | 35.81 ± 2.11 | 2.60 ± 0.57 |
| 1 | 1.7 | 4.00 ± 1.96 | 4.00 ± 2.00 | 0.00 | 1.00 ± 1.00 | 45.00 ± 4.97 | 28.00 ± 5.29 | 35.00 ± 5.92 | 5.00 ± 2.24 |
| 2 | 2.1 | 3.00 ± 1.71 | 0.00 | 0.00 | 3.00 ± 1.73 | 49.00 ± 5.00 | 18.00 ± 4.24 | 50.00 ± 7.07 | 5.00 ± 2.24 |
| 3 | 0.4 | 10.00* ± 3.00 | 4.00 ± 1.97 | 6.00* ± 2.39 | 2.00 ± 1.41 | 46.00 ± 4.98 | 29.00* ± 5.39 | 42.00 ± 6.48 | 8.00 ± 2.83 |
| 4 | 1.4 | 23.00* ± 4.21 | 10.00* ± 3.62 | 11.00* ± 4.69 | 14.00* ± 3.74 | 57.14 ± 6.36 | 25.71 ± 8.57 | 68.57* ± 14.00 | 31.43* ± 9.48 |
| 5 | 1.6 | 5.08 ± 2.86 | 1.69 ± 1.69 | 1.58 ± 1.69 | 1.69 ± 1.69 | 58.75* ± 5.50 | 25.00 ± 5.59 | 45.00 ± 7.50 | 3.75 ± 2.17 |
| 6 | 31 | 2.00 ± 1.40 | 1.00 ± 1.00 | 1.00 ± 1.00 | 0.00 | 51.00 ± 5.00 | 30.00* ± 5.48 | 33.00 ± 5.74 | 5.00 ± 2.24 |
| 7 | 19 | 6.00 ± 2.37 | 3.00 ± 1.73 | 1.00 ± 1.00 | 2.00 ± 1.41 | 48.67 ± 4.08 | 20.00 ± 3.65 | 27.33 ± 4.27 | 1.33 ± 0.94 |
| 8 | 14 | 8.00* ± 2.71 | 3.00 ± 2.23 | 2.00 ± 1.41 | 4.00 ± 2.00 | 60.00* ± 4.90 | 29.00* ± 5.39 | 47.00 ± 8.66 | 4.00 ± 2.00 |
| 9 | 32 | 2.00 ± 1.40 | 0.00 | 0.00 | 2.00 ± 1.41 | 45.00 ± 4.97 | 22.00 ± 4.69 | 34.00 ± 5.83 | 3.00 ± 1.73 |
| 10 | 1.0 | 2.44 ± 1.70 | 1.22 ± 1.22 | 0.00 | 1.22 ± 1.22 | 38.00 ± 4.85 | 11.00 ± 3.32 | 37.00 ± 6.08 | 0.00 |
| 11 | 14 | 3.00 ± 1.71 | 2.00 ± 1.41 | 0.00 | 1.00 ± 1.00 | 36.00 ± 4.80 | 15.00 ± 3.87 | 28.00 ± 5.29 | 1.00 ± 1.00 |
| 12 | 32 | 7.00* ± 2.65 | 0.00 | 0.00 | 7.00* ± 2.65 | 40.00 ± 4.90 | 17.00 ± 4.12 | 29.00 ± 5.39 | 0.00 |

Abbreviations: AM, aberrant metaphases; CsE, chromosome-type exchanges; CsF, chromosome-type fragments; CIA, chromatid-type aberrations. *Period between the end of therapeutic exposure and date when the blood sample was taken for this study. **Significantly different from control (p < 0.05).

reveal reliable differences from controls at any of the examined end points. One should remember that the patients examined had previously experienced therapeutic exposures and their cytogenetic characteristics may have been influenced by these exposures (4). Therefore, when a patient reveals an increased level of chromosome damage the main problem is to distinguish between the contribution of an enhanced individual radiosensitivity and the contribution of in vivo-induced lesions. This problem cannot be resolved in the present study, although some useful information can be obtained. Our next step will be to examine as a control group not healthy unexposed persons, but a group of therapeutically exposed patients with no late radiation ulcers.

For patients 6, 8, and 12, the influence of therapeutic exposure is unlikely, as these patients were exposed many years ago. It is generally accepted that lymphocytes with in vivo-induced chromosome lesions disappear from circulating blood with a half-life of approximately 3 years after exposure (5). Patient 5, though irradiated only 1.6 years ago, has normal spontaneous aberration levels, so the increased level of in vitro-induced damage probably reflects his elevated individual radiosensitivity. Patients 4 and 12 reveal not only an increased rate of chromosome-type aberrations, which are known to be the main result of in vitro exposures, but an increased rate of chromatid aberrations as well. This feature can be regarded as a peculiarity of their individual radiosensitivity. It is interesting to note that in all cases where the frequency of chromosome-type fragments in exposed cultures exceeds the control value (patients 3, 6, and 8), it is at the normal level in the unexposed counterparts. Thus it seems that the enhanced frequency of chromosome-type fragments presents chromosome response only to in vitro irradiation, without the contribution of therapeutic exposure.

Another approach to avoid the influence of therapeutic irradiation is to subtract (for each patient) the aberration level observed in unexposed culture from the level induced by 2 Gy and compare the result with the similar control value. The comparison of frequencies reduced by base levels renders the detected differences more reliable, but is less sensitive in detection of the differences, because relative errors of calculated reduced frequencies are higher than those of initial frequencies. This analysis corroborated the significant increase of aberrant metaphase frequency in patients 5 and 8, and of chromosome-type fragment frequency in patient 6. Other differences mentioned above, though still observed, were statistically insignificant (p > 0.05) using this comparison scheme.

If we regard the results for patients 5, 6, and 8 as the most reliable statistically and the results for patients 3, 4, and 12 as supportive, we come to the conclusion that at least some of the individuals with an elevated skin radiosensitivity also have an increased radiosensitivity of lymphocyte chromosomes.

The observed heterogeneity of chromosome radiosensitivity among the patients is not unexpected. In addition to individual variations in underlying sensitivity, variations can result from multiple causes including different time periods elapsed after in vivo exposure, different individual rates of elimination of aberrant lymphocytes from blood, errors in dose estimation during radiotherapy, imperfect statistical analyses applied, and other factors. The results described, obtained from a rather heterogeneous group of patients and still evidencing a correlation between two characteristics of radiosensitivity, appear encouraging for further research.

We regard the aberration pattern observed in cultures exposed in vitro as more suitable for evaluation of individual radiosensitivity than the aberration pattern in unexposed cultures because it appears that in vitro reaction is less dependent on the influence of in vivo exposures. The first argument favoring this suggestion concerns cellular distribution of aberrations. In healthy donors, aberrations are distributed according to Poisson distribution both in exposed (p = 0.8307) and unexposed (p = 0.8738) cultures. In patients, aberration distribution is very far from Poisson in unexposed cultures (p = 0.0009), which is characteristic of the effect of in vivo irradiation of a part of the body (6). In irradiated cultures, however, it again approaches the Poisson distribution (p = 0.0517), possibly hinting that previous in vivo exposure does not distort significantly the pattern of individual in vitro response. The second argument lies in the relative contribution of various aberration types in six patients selected as sensitive compared to the control pattern (data not shown). This comparison shows that in the unexposed cultures these patterns are completely different, implying different origin of spontaneous aberrations observed in the patients and in unexposed healthy persons. However, in the irradiated cultures the patterns observed in the patients closely resemble the controls. This indicates that when we compare aberration frequencies in irradiated cultures
of patients with the corresponding control values, we deal with phenomena of a similar nature, which makes the comparison more reasonable.

The kind of task we are performing in this study can be regarded as an approach to a more general, interesting, and topical problem—that is, an assessment of individual radiosensitivity. The variety of factors contributing to human radiosensitivity is extremely complex; obviously, it cannot be described with the aid of some single and general value. Its partial characteristics are numerous. As an indicator of human susceptibility to radiation we suggest the examination of the radiosensitivity of peripheral lymphocytes. This parameter is relevant to heredity and blood is radiosensitive and sensitive to alterations of the whole-body state and can be sampled readily in a noninvasive way. The results presented here, though preliminary, could be useful in this field of investigation.

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