Cytogenetic Biomonitoring of Human Radiation Exposures: Possibilities, Problems and Pitfalls

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Chromosome aberrations/Monitoring/Low dose/Stress response/Inverse dose-rate effect

The use of chromosome aberration analysis has progressed and is now generally recognized to provide a useful means for the assessment of dose and possible health consequences in human exposures to relatively high doses of ionizing radiations. The automated analysis of radiation-induced chromosome aberrations will facilitate the better understanding of the health effects of radiations on human populations, particularly of those at low levels. However, the reaction kinetics of chromosome aberrations in the low dose or low dose-rate exposures are not well delineated. In this paper, possible reaction kinetics of chromosome aberrations, particularly those in human lymphocyte, in low dose-rate exposures have been discussed based on the cytogenetic data on the Thorotrast patients, persons involved in the protracted occupational exposures and modulation of chromosome aberrations by radioadaptive response. In the protracted low dose-rate exposures, the levels of chromosome aberrations, whether they are stable- or unstable-type aberrations, are not in a simple function of the total accumulated dose due mainly to the lymphocyte kinetics and modulation by cellular stress response. If this formalism is realistic, the dissociation between levels of chromosome aberrations and cancer risks may be large for the low level protracted exposures.

INTRODUCTION

Quantitative measurement of biological responses to ionizing radiation is clearly of the utmost importance in regard to the radiation protection and risk assessment in human radiation exposures. Since the first attempt by Bender and Gooch in 1966 in persons involved in the Recuplex accident, chromosome aberrations in the peripheral blood lymphocytes have been demonstrated and now generally accepted to provide the most reliable measure of radiation dose to man, so far we are concerned with the acute exposures to relatively high doses.

However, our recent concern has shifted to the chromosomal responses to the low level exposures and their biological meaning. This is really the area where the development of automated chromosome analysis is encouraged, and at the same time, the reaction kinetics of chromosome aberrations are needed to be elucidated for the implication of the quantity and quality of chromosome aberrations. Since the use of chromosome aberration analysis for biological dosimetry and its laboratory procedures are seen in the recent comprehensive reviews, I take this opportunity to give some considerations on the reaction kinetics of chromosome aberrations in low dose-rate exposures.
CHROMOSOMAL RESPONSE TO THE SINGLE IONIZING PARTICLE

In the low dose-rate exposures, the incident flux to the cell is usually one ionizing particle, including photon from X- or γ-rays, per cell. Therefore, the mean dose to the affected cells can not be below the dose given by a single path of ionizing particle. This dose is called elementary dose\(^4\), and is dependent on the quality of radiations. With the increase of the exposure dose, the dose to the cell stays constant and only the fraction of affected cells increases in the low dose range, then the cellular dose increases in the higher dose range.

For the lymphocyte nucleus with 5 μm diameter, the elementary dose to the cell nucleus from γ-ray, i.e., average dose given to a cell by a single path of γ-ray photon, is about 0.2 cGy. Taking 2–2.5×10\(^{-4}\)/cGy for the rate of induction of dicentrics by γ-rays\(^5\), the probability for the affected cell to have dicentrics is nearly 5×10\(^{-5}\), which is obviously beyond the practical analysis for chromosomal response to the elementary dose. The elementary dose is dependent on the quality of radiation. For instance, that for 5 MeV α-particle is about 70 cGy. This is high enough for the analysis of chromosomal reaction to the single path of ionizing particle.

With this reasoning, we selected persons who had been injected with Thorotrast. Thorotrast is a colloidal preparation of \(^{232}\)Th, and has been used as an X-ray contrast medium in the early of 1900's. Once injected, it is excreted at a negligible rate, taken up by reticuloendothelial cells and continues to irradiate the neighboring cells. The radiations involved are α-, β- and γ-rays, but about 95% of the dose is due to α-particles (4.07–8.95 MeV). We have studied the chromosome aberrations in peripheral blood of 63 such persons\(^6\). Table 1 summarizes the results of chromosome analysis. Unlike acute whole body exposures or partial body exposures, the distribution of chromosome aberrations does not conform to the Poisson or 0-truncated Poisson distribution.

But, instead, these distributions of chromosome aberrations are best fitted by the following formula.

Table 1. Summary of chromosome aberrations in blood lymphocytes from Thorotrast patients.

| μ  | No. of patients | No. of cells | Distribution of cells with indicated no. of dic. + rings | Mean (m) | Goodness of fit (p) to Present model |
|----|-----------------|--------------|--------------------------------------------------------|---------|-----------------------------------|
|    |                 |              | 0 | 1 | 2 | 3 | 4 | 5 | Poisson | 0-truncated Poisson |
| 0–0.04 | 28 | 7,507 | 7,356 | 111 | 31 | 8 | 1 | 0 | 0.027 | <0.001 | 0.804 | 0.018 |
| 0.05–0.09 | 13 | 4,201 | 4,013 | 114 | 45 | 25 | 4 | 0 | 0.070 | <0.001 | 0.109 | 0.108 |
| 0.10–0.14 | 9 | 4,483 | 4,125 | 210 | 92 | 39 | 13 | 4 | 0.130 | <0.001 | 0.254 | 0.670 |
| 0.15–0.19 | 8 | 3,233 | 2,890 | 203 | 88 | 33 | 14 | 5 | 0.174 | <0.001 | 0.065 | 0.884 |
| 0.20–0.24 | 1 | 500 | 433 | 39 | 20 | 5 | 2 | 1 | 0.214 | <0.001 | 0.071 | 0.923 |
| 0.25–0.29 | 2 | 750 | 640 | 62 | 27 | 12 | 5 | 4 | 0.259 | <0.001 | 0.057 | 0.816 |
| 0.30–0.40 | 2 | 700 | 575 | 63 | 37 | 12 | 7 | 6 | 0.333 | <0.001 | 0.033 | 0.756 |

Total | 63 | 21,374 | 20,032 | 802 | 340 | 134 | 46 | 20 | 0.102 |

μ: Mean aberration frequency in each patient.
The parameters are:

- \( \lambda \): rate of cells to be lost either by death or cell division.
- \( \mu \): mean frequency of aberrations as expressed on a per-cell basis.
- \( A \): mean number of \( \alpha \)-particles passing through a cell nucleus per unit time.
- \( k \): number of paths of \( \alpha \)-particle.
- \( a \): production coefficient for chromosome aberrations.
- \( S \): survival (interphase death) after a single path of \( \alpha \)-particle.

The rational of this model is that the lymphocytes are continuously replenished according to their life-span, and the age distribution of lymphocytes is approximated by \( F(t) = \lambda e^{-\lambda t} \), where \( 1/\lambda \) is the mean lifetime of lymphocytes. Therefore, the longer-lived lymphocytes might have the more chance to be hit.

Since \( \mu \) is known for each person, the production coefficient \( a \) can be estimated by the maximum likelihood method that satisfies the Equation (1) for the distribution of aberrations in all persons. Thus, \( a = 0.81 \pm 0.03 \) was obtained, implying that approximately 0.8 dicentrics and rings are produced by a single path of \( \alpha \)-particle.

Fig. 1 depicts the relationship between mean aberration frequency, \( \mu \), and cell survival, \( S \). It is evident that if \( S \) is close to the unity, the mean aberration frequency is simply proportional to the fluence rate (dose rate) as expressed by \( aA/\lambda \). However, when \( S \) is smaller than the unity, the mean aberration frequency increases with the increase of fluence rate but soon reaches saturation.

**Fig. 1.** The relationship between the mean aberration frequency \( (\mu) \) and fluence rate to lymphocyte \( (A) \) in protracted exposure to radiation. \( S \) is lymphocyte survival after single path of ionizing particle. At \( S = 1 \), the aberration frequency is expressed by \( aA/\lambda \), while at \( S < 1 \) the yield saturates at \( aS/(1-S) \).
a plateau level where it remains constant against further increase of fluence rate. The plateau level is dependent on the probability of cell killing, $S$, and induction efficiency, $a$. Therefore, as regard to the chromosome-based biological dosimetry, it should be noted that in case of protracted exposure, the level of unstable aberrations is not a reflection of total accumulated dose. In the exposures to low LET radiations, where $S$ is close to 1, the aberration frequency is a reflection of dose-rate, and in the exposure to high LET radiations, where $S$ is smaller than 1, the level of aberrations is the reflection of the quality of radiations. This may be the major reason for the lack of dose-response relationship between chromosome aberration frequency in lymphocytes and body burden of Thorotrast\textsuperscript{6–8}. In the in vitro experiment using human lymphocytes, $S=0.34$ has been obtained for 4.9 MeV $\alpha$-particles\textsuperscript{9}. Taking the values of $a=0.8$ (aberrations/path), $S=0.34$ and $1/\lambda=1500$ days, an average fluence rate of $A=1.5$ hits (1.04 Gy)/cell/year is obtained.

Fig. 2 shows the chromosome aberration frequencies in German Thorotrast patients reported by Kemmer et al.\textsuperscript{8}. Again, it is evident that the aberration frequencies are compatible with the Equation (1); they are neither in a simple function of dose-rate nor of the total accumulated dose. The aberration frequencies stay at a level of about 0.9 dicentrics per cell. This value at the plateau level gives $A=1.54$ for an average number of hits per cell per year, which is very comparable to that obtained for the Japanese patients ($A=1.5$).

![Graph](image-url)  
**Fig. 2.** The frequencies of dicentrics against accumulated dose from internal deposit of Thorotrast (Reproduced from Kemmer et al.\textsuperscript{8}). The total accumulated doses are expressed by $^{224}\text{Ra}$ equivalent activity ($10^{-9}$Ci) multiplied by time of exposure (years).
REACTION KINETICS FOR LOW DOSE-RATE OF LOW LET RADIATIONS

From Fig. 1, it is assumed that the aberration frequency may saturate at $\mu = aA/\lambda$ for the protracted exposures to low LET radiations, such as X- or $\gamma$-rays because the cell killing by an elementary dose is negligible ($S=1$).

For the unstable aberrations, they may be formed at a rate of $aA$, but at the same time they may be eliminated according to the lymphocyte lifetime. Therefore, the frequency of aberrations at time $t$ from the commencement of exposure will be expressed by

$$
N_u = \frac{aA}{\lambda} (1 - e^{-\mu t}), \quad \text{or} \quad N_u = \frac{aA}{\lambda} (1 - e^{-\lambda T/A}), \quad \text{..................................... (2)}
$$

where $T$ is the total accumulated dose. If $t$ is long enough, the aberration frequency is expressed by $N_u = aA/\lambda$, implying that the aberration frequency is not in a function of accumulated dose but in a function of dose rate. Fig. 3 shows the results of our chromosome analysis in the peripheral blood lymphocytes based on 230,717 cells from 931 radiological technicians who were occupationally exposed to low-level X- and $\gamma$-rays. The observations are consistent with the above model of the reaction kinetics. If we apply the mean dose rate of 1.5 cGy per year, $a = 4.0 \times 10^{-4}$/cGy is obtained for the induction coefficient, which is comparable to those obtained in the in vitro experiments ($2-2.5 \times 10^{-4}$/cGy$^5$).

In contrast to the unstable aberrations, the cells with only stable aberrations, Cs cells, have no reason to be lost when they attempt at cell division. Such aberrations may be inherited by
daughter cells during the replenishment of lymphocytes. Therefore, the level of these cells are expected to be simply proportional to the accumulated dose as expressed by

$$N_s = bA_t, \quad \text{or} \quad N_s = bt$$

where the parameter $b$ is a production coefficient of such cells.

As seen in Fig. 3, the observations on the Cs cells are not in agreement with such expectation. The frequency is rather refractory to the accumulated dose, staying constant or increases with very weak dependence on the dose accumulation. This contrast to the observations in A-bomb survivors where clear dose-response relationships have been observed. The difference can not be simply explained by the difference in the dose rate. In this connection, it should be noted that the target cells for their induction are different between Cu and Cs cells. Because of their unstable nature, dicentrics and rings that we are analyzing are those induced in the terminal cells; they were either in the terminal stage of $G_1$ or in $G_0$ stage at the time of exposure and have never experienced cell division thereafter. However, the majority of Cs cells are assumed to be cycling cells or their precursor cells at the time of irradiation but recovered at $G_0$ stage by sampling. The difference in the cellular stages at the time of irradiation may be relevant to this discrepancy (see below).

MODULATION BY ADAPTIVE RESPONSE

Recently, evidence has been accumulated indicating that the small dose of low LET radiation makes the cells less susceptible to the chromosome aberration formation by subsequent exposures to higher doses. The phenomenon is now referred to as "radioadaptive response" named after the adaptive induction of DNA-repair pathway in $E. \ coli$ treated with non-toxic level of alkylating agents.

Since X- or $\gamma$- rays at dose as low as 1 cGy is sufficient for the induction of this response, it is likely that the cell is responding to a single energy loss event, and also that the cellular target is not necessarily the cell nucleus but is likely to be the cell as a whole or the cell membrane. Unlike adaptive response to alkylating agents in bacteria, the response observed in mammalian and other eukaryotic cells shows a wide variation of cross-resistance. This indicates that the response is a cellular manifestation of the consequences of more general form of stress response of the cells.

The adaptive response can be observed for the low LET radiation at a dose up to about 10 cGy, and interestingly it is less efficient for a single shot of high LET radiations. Probably the elementary dose may be too high for the adaptive response in the high LET radiations. In our experiment using human lymphocytes, the adaptive response was observed only in the PHA-stimulated $G_1$ lymphocytes but not in the unstimulated $G_0$ cells. This stage difference in the response might be relevant to the lack of clear dose response relationship of the Cs cells in the radiological technicians.

Such stress response may be a factor which modulates the dose-response relationship of variety of radiation-induced biological consequences. During protracted exposures with suf-
ficiently low dose-rate, the energy loss events are sequential. The biological effects may be modulated when the ionization occurs in the cells which are in an active state of stress response. There are considerable evidence indicating that the mutational, including chromosomal, events are suppressed to occur in the cells in stress response\textsuperscript{11}. Interesting to note is that such stress response is likely to be the early cellular response which occurs when the cells are confronted to some environmental insults. Among many others, the up-regulation of the activation of protein kinase C, the expression of c-fos and c-jun protooncogenes have been demonstrated\textsuperscript{15-17}. The enhanced expression of DNA repair systems has been postulated to account for the suppression of mutational events by stress response.

Let's assume that the cells are continuously exposed to low doses of mixed $\alpha$- and $\gamma$-rays. The cells hit by $\gamma$-ray photons will show adaptive response. The mutagenic response of the cells to the $\alpha$-particles may be different between the cells which are hit by $\gamma$-rays previously and hence in adaptive condition and those which are either not hit by $\gamma$-rays or run out of the adaptive condition. The mutagenic yield is expressed by

$$ Y = a(nS) + \beta(nS)^2 $$

in the non-adapted cells, and

$$ Y' = a(nRS') + \beta(nRS')^2 $$

in the adapted cells. And, therefore, in the steady state of continuous exposure, the over-all mutation frequency by $\alpha$-particles in the living cells is expressed by

$$ MF = P_a \sum Y + P_a' \sum Y' $$

where $n$ is the number of $\alpha$-particles passing through the cell nucleus, $R$ is the dose modification factor in the adapted cells, $S$ is cell survival after a single path of $\alpha$-particle in non-adapted cells, and $S'$ is that for adapted cells as expressed by $S' = Se^{-(1-R)(\ln S)}$. $P_a$ and $P_a'$ are the fractions of non-adapted and adapted cells in which the cell nucleus is hit by $\alpha$-particle, respectively. The non-adapted cells are either those not hit by $\gamma$-rays, or those in which the adapted condition is canceled by a hit by $\alpha$-particle or by an accumulation of $\gamma$-ray dose (in this case, rate of retention of adaptive condition in a function of $\gamma$-ray dose is tentatively set at $D_{10} = 10$ cGy). It is also assumed that the fraction of the adapted cells is influenced by the cell-to-cell communication of stress signals. If we assume that the contaminated $\gamma$-rays constitute 100% of the total dose and then replace $\alpha$-particles with photons, the same model can be applied for the continuous $X$- or $\gamma$-ray exposure.

Fig. 4 shows the change in the biological consequences against the dose-rate which is expressed by a dose in a unit time, $T$. The time limitation, $T$, is the persistent time of adapted condition. In our estimate in the PHA-stimulated human lymphocytes, $T$ was about 9 hours\textsuperscript{18}. As seen in Fig. 4, the model predicts that the mutational events decrease with the decreasing dose-rate and then again increase with further decrease of the dose rate showing inverse dose-rate effects in the very low dose-rate range. Then inverse dose-rate effects are due to the non-randomness of the hits by $\gamma$-ray photons.

While the mutagenic events are also involved in the malignant transformation of the cells,
the expression of the malignant phenotype is assumed to be modulated by many other cellular factors. During the pathway of stress response, the PI turnover product, diacylglycerol, which activate protein kinase C, is known to be analogous in its function to the tumor promoter, phorbol ester such as TPA. It is therefore tempting to assume that the adaptive condition promotes the "expression" of the carcinogenic mutations while the mutation production itself is suppressed to occur. Hill et al. reported that the neutron-induced malignant transformation in vitro was about 8 times higher in the cells treated with TPA. If we incorporate this dose-modification factor for the malignant transformation of the cells in stress response to y-rays, we can expect the inverse dose-rate effect of malignant transformation as presented in Fig. 4. The inverse dose-rate effect of malignant transformation is different from that for mutation induction; the inverse dose-rate effect for malignant transformation is due to the enhanced expression of carcinogenic mutation in the adapted cells and occurs in the higher dose-rate range than that for the expression of mutations or chromosome aberrations. The inverse dose-rate effect for malignant transformation has been noted in the irradiation of fission neutrons which are usually contaminated with y-rays while it is absent or less pronounced for irradiation by a-particles relatively free of y-ray contamination.

In the low dose-rate exposures, much is remained to be elucidate for the implication of the levels of chromosome aberrations. However, as predicted in the present formalism, it is noteworthy that there is a considerable dissociation between mutational, including chromosomal, events and the expression of malignant phenotype. The present model poses a warning against the use of chromosome aberration frequencies in the risk assessment of cancer development in the protracted low dose-rate exposures.
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