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Perspective

Practical applications of cytogenetic biodosimetry in radiological emergencies

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BACKGROUND

The Fukushima nuclear disaster caused by the earthquake and tsunami on March 11, 2011 is believed to be the worst nuclear accident since the Chernobyl disaster in 1986. The Korean government is providing support to Japan and is also trying to protect the people from the hazards of ionizing radiation. To this end, Korean rescuers have been dispatched to the disaster-struck areas to assist in the relief operations.

Our institution, National Radiation Emergency Medical Center (NREMC), has been providing medical services to the people returning from the contaminated areas of Japan. The NREMC performs various medical activities in cases of radiological emergencies, including assessment of the absorbed dose in cases of suspected irradiation. Although physical dosimetry, like a personal badge, is routinely used in pre-arranged works in the contaminated areas, most people who were examined in Fukushima did not carry any type of physical dosimeters. Thus, the main issue was to determine whether these people were overexposed to radiation.

Cytogenetic biodosimetry is one of the methods that can be used to retrospectively assess the irradiated dose. However, the indications for this approach need to be understood

adequately and the results must be interpreted carefully. As a neighboring country, we were highly impacted by the nuclear crisis. However, we can learn from such disasters and prepare ourselves to face such crises in the future. I hope that this article helps people understand the usefulness and limitations of cytogenetic biodosimetry in radiological emergencies.

WHAT IS THE PURPOSE OF A BIOLOGICAL DOSE ASSESSMENT?

Biological dose assessment by biodosimetry is based on the analysis of biomarkers that reflect the damage caused by ionizing radiation. These biomarkers include lymphocyte counts, dicentric or ring chromosomes, and other types of chromosomal aberrations. Biodosimetry is an important, and in some cases, the only source of information for the investigation of radiological emergencies, because patients with suspected overexposure usually do not wear personal dosimeters during actual accidents [1].

In cases of high exposure, dose assessment facilitates planning of the treatment and alerts physicians to the possible health consequences. In cases of low-dose exposure (exposure below the level where treatment is required), dosimetric information is essential for physicians while counseling the patients about health risks.

APPLICATION OF DOSE CONCEPTS IN BIOLOGICAL DOSIMETRY

1. Number of lymphocytes in the peripheral blood

Healthy adults usually have lymphocyte counts in the range of $1.5-4.0 \times 10^9/L$ of whole blood. In cases of high-dose irradiation with a few Gy (gray units), one of the early deterministic reactions is a rapid decrease in the number of lymphocytes (lymphocytopenia) in the peripheral blood. Lymphocytopenia occurs before the other forms of cytopenias



(granulocytopenia and thrombocytopenia), and it occurs when the energy transmitted by the ionizing radiation damages the hematopoietic cells, within the first 6-24 hours of an exposure. The main advantage of counting the number of lymphocytes is the simplicity of this method. The turn-around-time for counting lymphocytes is less than an hour in most laboratories. Table 1 shows the association between the lymphocyte count and the expected absorbed doses on the basis of the severity of the clinical symptoms [2].

However, since the number of lymphocytes is hardly affected by a dose less than 1 Gy, it is difficult to detect low-dose exposures.

2. Construction of a dose-response calibration curve

Because of inter-laboratory technical differences, laboratories that intend to perform biodosimetry should construct their own dose-response curves [1]. To construct a calibration curve, lymphocytes are irradiated *in vitro* while simulating the *in vivo* situation as closely as possible. In this process, freshly obtained whole blood specimens in lithium (or sodium) heparin are irradiated at 37°C, with the sample placed far away from the source to ensure uniform irradiation. For example, if the specimen tube is 0.01 m in diameter, it should be placed at least 1 m away from the source to minimize the variation between the closest

and the farthest lymphocytes (the variation in this case will be less than 2%). There is strong evidence that the yields of dicentric chromosomes (Y) produced by low linear-energy-transfer (LET) radiation are related to the dose (D) by the linear quadratic equation:

$$Y=C+\alpha D+\beta D^2$$

Since curve-fitting is based on Poisson statistics, the dicentric cell distribution for each dose should be tested for its compliance with the Poisson distribution. The most commonly used method in such assessments is the μ test; μ values higher than 1.96 indicate overdispersion. Underdispersion, with μ values lower than 1.96, is very unlikely to occur biologically and may indicate a discrepancy in the data. To minimize errors in plotting the curve, 10 or more doses in the range of 0.25-5.0 Gy should be used. Since many radiation accidents involve doses less than 1.0 Gy, the lower end of the curve is particularly important. At least 4 points in the range of 0.25-1.0 Gy should be included, and inclusion of a dose below 0.25 Gy is highly desirable. For low LET radiation, it is not necessary to have a point higher than 5.0 Gy. Table 2 shows the doses used in the dose-response curve constructed at NREMC and the yield and distribution of dicentric cells. The standard curve with the standard error is shown in Fig. 1.

Ideally, a laboratory should also generate its own background data. A consensus has emerged that the background level of dicentrics is about 0.5 to 1.0 per 1,000 cells. Several software programs, including R software and BIDOSE, are available for proper curve-fitting.

ARE CHROMOSOME ABERRATIONS SPECIFIC TO IONIZING RADIATIONS?

Dicentric chromosomes are known to be specific biomarkers for ionizing radiations [3, 4]. However, some chemotherapeutic agents like bleomycin and mitomycin C can cause similar damage to the chromosomes. Reciprocal translocation can be caused by low-dose chronic exposure and can pass successfully through cell cycles. Thus, it can be

Table 1. Relationship between the absolute lymphocyte count and the absorbed dose 6 days after the first exposure (IAEA and WHO, 1998).

Acute Radiation Syndrome (ARS)	Absorbed dose (Gy)	Number of lymphocytes (per μ L of the whole blood)
No symptoms	0.1-1.0	1,500-2,500
Mild	1.0-2.0	700-1,500
Moderate	2.0-4.0	500-800
Severe	4.0-6.0	300-500
Very severe	6.0-8.0	100-300
Lethal	> 8.0	0-50

Table 2. Yield and the distribution of dicentric chromosomes for each radiation dose in the standard dose-response curve at NREMC.

Dose (Gy)	Cells analyzed	Distribution of dics ^{a)}						Distribution of trics ^{b)}		Sum of dics	Yield of dics (Y)
		1	2	3	4	5	6	1	2		
0.00	10,000	15								15	0.0015
0.10	2,000	9								9	0.0045
0.25	2,000	24								24	0.0120
0.50	2,000	57	3							63	0.0315
0.75	2,000	112	3							118	0.0640
1.00	2,000	195	10	1						218	0.1090
2.00	700	180	24	2				2		238	0.3400
3.00	400	154	43	13	1			4		291	0.7275
4.00	200	78	54	12	2	1		6	1	251	1.2550
5.00	200	71	55	29	15	2	1	14	2	380	1.9000

Abbreviations: dics, ^{a)}dicentric chromosomes; trics, ^{b)}trivalent chromosomes.

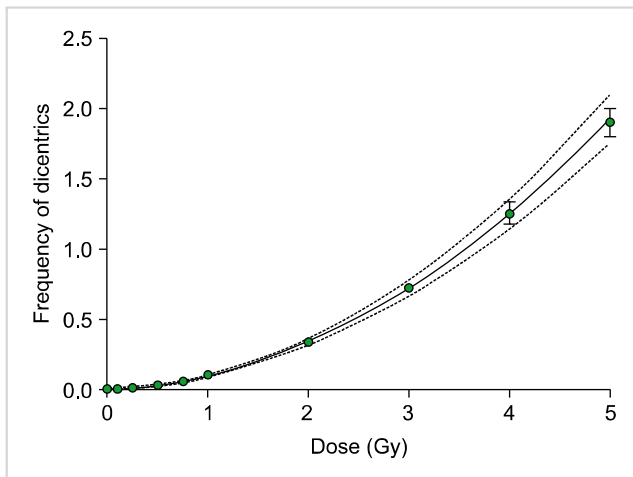


Fig. 1. The dose-response curve representing the estimated dose with standard error as constructed by NREMC.

an indicator of the cumulative effect of various environmental toxigens and can represent individual specific variation in radiosensitivity, which may be influenced by the age of individuals, habits such as smoking and alcohol consumption, and polymorphisms in the DNA repair genes.

When acute exposure to ionizing radiation is suspected, the first step is to check whether the yield of dicentric chromosomes is significantly higher than the expected background level; therefore, the normal background levels need to be analyzed carefully and they depend on at least 2 factors: the biodosimetry method used in every laboratory and the local natural background radiation level, which probably effects a spontaneous increase in the rate of dicentric. The background radiation level has been accepted to be 0.5-1.0 per 1,000 cells; the highest level of 2.99 per 1,000 cells was reported by Ganguly. In our study at NREMC, 15 dicentric were found in 10,000 cells in 10 normal subjects (1.5 per 1000 cells).

HEALTH EFFECTS OF CHROMOSOMAL ABERRATIONS

The mechanism underlying the adverse health effects after exposure to ionizing radiations has not been fully understood. Ionizing radiations can affect the DNA or chromosomes. While unstable aberrations such as dicentric or ring chromosomes are unlikely to pass through a cell cycle, cells with stable aberrations like reciprocal translocations or insertions do not undergo negative selection during mitoses because they are not very harmful. However, persistence of stable aberrations is known to be a risk factor for developing cancer after a few years.

The Radiation Effects Research Foundation (RERF) in Japan followed up with the survivors of the Hiroshima and

Nagasaki bombings for more than 50 years and found that occurrence of solid cancers increases in proportion to the radiation dose. The BEIR VII report suggested the “linear no-threshold” (LNT) model, which states that the risk would persist in a linear fashion at lower doses without a threshold; thus, every exposure holds some risk, which is usually proportional to the dose [5].

CONCLUSION

Cytogenetic biodosimetry, which is complementary or an alternative to physical dosimetry, is a useful tool for evaluating the absorbed dose of ionizing radiation. The assay for dicentric chromosomes of circulating lymphocytes is currently the gold standard and should be a method of choice when most of the body is exposed to acute radiation in an accident. However, if the exposure involves only a small portion of the body excluding a significant portion of the bone marrow, lymphocyte analysis would yield limited data. Moreover, the biologically estimated dose is not directly measured but is calculated statistically, and hence, it may disagree with the physically measured dose.

In cases of low-dose or chronic exposure, all the available information should be collected and combined with the data from biological dosimetry in order to understand the case correctly. Information about the events, such as the distance from the radiation source, may be required and can be obtained by questioning patients and witnesses. Other information may be obtained from the clinical signs and symptoms of the patient and also from the physically measured doses in the irradiated individuals. Scientists around the world are trying to improve the biodosimetry system to make it faster and more accurate by using various biomarkers.

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