J. RADIAT. RES., 42: SUPPL., S149–S155 (2001)

Cytogenetical Dose Estimation for 3 Severely Exposed Patients in the JCO Criticality Accident in Tokai-mura

ISAMU HAYATA[1]*, REIKO KANDA[1], MASAKO MINAMIHISAMATSU[1]
AKIRA FURUKAWA[1] and MASAO S. SASAKI[2]

[1]National Institute of Radiological Sciences, 4–9–1 Anagawa, Inage-ku,
Chiba 263–8555, Japan
[2]Radiation Biology Center, Kyoto University, Yoshida-konoecho,
Sakyo-ku, Kyoto 606–8501, Japan

Criticality accident/Dose estimation/Dicentrics/Rings/Premature chromosome condensation

A dose estimation by chromosome analysis was performed on the 3 severely exposed patients in the Tokai-mura criticality accident. Drastically reduced lymphocyte counts suggested that the whole-body dose of radiation which they had been exposed to was unprecedentedly high. Because the number of lymphocytes in the white blood cells in two patients was very low, we could not culture and harvest cells by the conventional method. To collect the number of lymphocytes necessary for chromosome preparation, we processed blood samples by a modified method, called the high-yield chromosome preparation method. With this technique, we could culture and harvest cells, and then make air-dried chromosome slides. We applied a new dose-estimation method involving an artificially induced prematurely condensed ring chromosome, the PCC-ring method, to estimate an unusually high dose with a short time. The estimated doses by the PCC-ring method were in fairly good accordance with those by the conventional dicentric and ring chromosome (Dic+R) method. The biologically estimated dose was comparable with that estimated by a physical method. As far as we know, the estimated dose of the most severely exposed patient in the present study is the highest recorded among that chromosome analyses have been able to estimate in humans.

INTRODUCTION

Chromosome aberrations in the peripheral lymphocytes are the most reliable indicator in a biological dose estimation. In the conventional method for radiation dosimetry, we score marker aberrations, such as dicentric and ring chromosomes as well as fragments among chromosomal aberrations in the lymphocytes, and estimate the dose. However, when the exposed dose is high, it becomes difficult to apply this method, because not only the number of the lymphocytes in blood cells is reduced, but also the cell cycle is delayed to reach the metaphase. Therefore, it is not possible to make a chromosome preparation containing a sufficient number of metaphase cells necessary for a dose estimation, as routinely done in cytogenetical dosimetry.

*Corresponding author: Phone; +81–43–206–3080, Fax; +81–43–206–3080, E-mail; hayata@nirs.go.jp

In the criticality accident in Tokai-mura that occurred on September 30, 1999, the three workers at the site were severely exposed to neutrons and γ-rays. The drastically reduced number of the lymphocytes indicated that the whole-body dose of radiation which they had been exposed to was unprecedentedly high. We thus applied a high-yield chromosome preparation method[1)] to prepare chromosome slides. This method concentrated the lymphocytes, increased the recovery rate of the sedimentary cells by centrifuging, and made the frequency of analyzable cells very high. In this way, we could obtain a sufficient number of analyzable cells from 4 ml of blood, even in a metaphase sample 48 hours after the accident. For a chromosome analysis we applied two different methods: a) the new dose-estimation method involving an artificially induced prematurely condensed ring chromosome (PCC-ring)[2)] and b) the standard method utilizing dicentric and ring chromosomes (Dic+R). The analysis by the PCC-ring method gave a rough, but quick, estimation of the doses 53.5 hours after the first blood collection. Although the analysis by Dic+R frequency was very time-consuming and technically very challenging, it gave accurate estimations of the doses. It also confirmed that the doses obtained early by PCC-ring were reliable.

The present report describes a cytogenetical dose estimation performed on 3 severely exposed patients, whom we refer here as A, B and C in order of severity.

MATERIALS AND METHODS

Three male patients were transported to the National Institute of Radiological Sciences 5 hours after the accident. Peripheral blood for chromosome analyses was obtained 3 times: 9 hours, 23 hours and 48 hours after the accident. The fraction of lymphocytes in the white blood cells in the 9 hour-samples of the patients was already reduced to as low as 1.9% (A), 2.1% (B) and 15% (C), respectively. The corresponding normal values are between 25–48%. The absolute number of lymphocyte counts of the most severely exposed patient A became zero on the third day after the accident.

Chromosome preparations were made according to our improved methods[1,2)] for high-dose exposure cases as follows. About 8 ml of the peripheral blood was obtained each time from each patient. Lymphocyte-rich mononucleated cells, separated with VACUTAINER CPT tubes (Becton Dickinson Co., Ltd.), were mixed with 12 ml of culture medium consisting of 9.6 ml of a RPMI 1640 solution, 2.4 ml of calf serum, 0.72 mg of Kanamycin, and 0.24 ml of PHA, and then divided into two 15 ml centrifuge tubes. One tube was processed for the preparation of scoring Dic+R, and the other was used for a PCC-ring analysis. Both of them were cultured in the CO_2 incubator for 48 hours at 37°C. The former was supplemented with 0.3 μg Colcemid at the start of the culture. The latter was treated with 500 nM Okadaic acid for the last one hour of the culture. The cultured cells were collected by centrifugal sedimentation, treated with a 0.075 M KCl hypotonic solution for 20 minutes at 37°C, fixed with 1:3 acetic alcohol three times, and stored at –20°C in the freezer for more than 3 hours. Air-dry slides were made in a warm and humidified box[3)] and densely stained with Giemsa's solution.

Chromosome aberrations were scored under a microscope equipped with an automated stage

controlled by a computer. All locations of the analyzed metaphase cells on a slide were recorded by computer and the cells including normal cells were repeatedly examined. All of the cells carrying the aberrations were photographed. A multicentric chromosome with n centromeres was counted as n-1 dicentric chromosomes.

The dose was estimated in equivalent dose to ^{60}Co γ-rays or to X-rays, whose radiation quality factor was considered to be unity. A dose (GyE') estimation by the PCC-ring was made by comparing the observed data with the experimentally obtained values in the dose response of the PCC-ring against 200 kV X-rays[2]. A dose (GyE) estimation by the dicentrics and rings in patient C was made according to the standard method shown in IAEA Technical Report Series No. 260[4]. The reference we used for the dose-response of the dicentric and ring chromosomes accompanied by a fragment (Dic+Rc) in the human lymphocytes was that of ^{60}Co γ-rays: $Y = (2.31 \pm 0.88) \times 10^{-2}D + (6.33 \pm 0.25) \times 10^{-2}D^2$ (Sasaki, unpublished), where Y equals the number of Dic+Rc per cell and D equals the dose in Gy. The radiation doses of patients A and B were higher than the dose that can be estimated by a linear quadratic calculation formula[5]. Because the number of dicentric chromosomes saturates at doses above 6 Gy, due to the limited number of human chromosomes[6], i.e., the theoretical maximum number of dicentric chromosomes is 45 (46-1) in humans. Therefore, those doses (GyE'') were estimated by a direct comparison of the observed frequencies with those obtained in a study of 1.9 MeV X-rays (equivalent to γ-rays) by Norman and Sasaki (1966)[6].

RESULTS AND REMARKS

In the context of the need for immediate planning of clinical treatment, it is essential to estimate the exposed dose as quickly as possible, especially in the case of an accident involving high dose. Therefore, we first performed a PCC-ring analysis, by which we could quickly estimate, though crudely, the radiation dose (Fig. 1). It was possible by PCC-ring scoring to estimate the doses of all 3 patients 53.5 hours after the first blood collection. The frequencies of PCC-ring per 100 cells in the samples obtained 9 hours after the accident were 150, 77, and 24, respectively. The doses estimated from those PCC-ring values were higher than 20 GyE' (Gy equivalent to 200 kV X-rays) for patient A, 7.4(6.5–8.2) GyE' for patient B, and 2.3(1.8-2.8) GyE' for patient C (Fig. 2).

Regarding dicentric and ring chromosomes, it was rather easy to score the chromosome aberrations in patient C, while the scoring was very difficult in the other two patients due to the low mitotic index. In the samples of patients B and C, however, the morphology of chromosomes in each cell was sufficiently good to detect the abnormal chromosomes (Fig. 3). On the other hand, in the sample of patient A it was not rare to find a chromosome with several centromeres, but where the exact number of centromeres was difficult to determine (Fig. 4). There were also many fragments in this sample, and it was not always possible to confirm all 46 centromeres per cell. Therefore, we counted conservatively the number of centromeres that could be positively identified in the abnormal chromosomes. This might lead to minimize the number of dicentric chromosomes, and thus the estimated dose of patient A would be lower than the dose he had

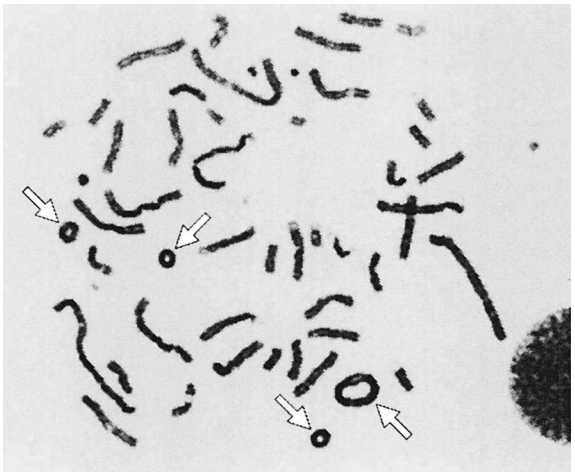

Fig. 1. Prematurely condensed chromosomes having PCC-rings (white arrows) in a lymphocyte of patient A.

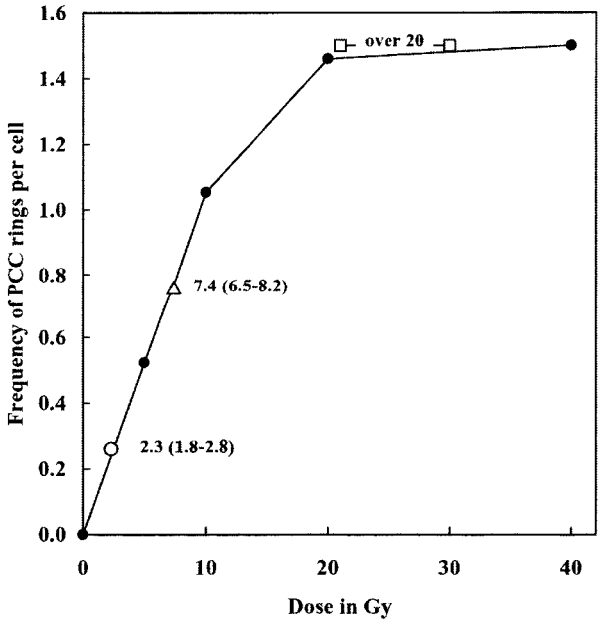

Fig. 2. Dose-response curve of the PCC-ring for 200 kV X-rays and the estimated doses of patients A(□), B (△) and C(○).

actually received. Ring chromosomes with a centromere as well as those without a centromere were mixed and counted as ring chromosomes in patient A. The result of scoring dicentric chromosomes (Dic), ring chromosomes with a centromere (Rc), and ring chromosomes with/without a centromere (R) is summarized in Table 1.

Since in each patient the frequencies of chromosome aberrations were not significantly different among the samples obtained 9, 23 and 48 hours after the accident, the results of these

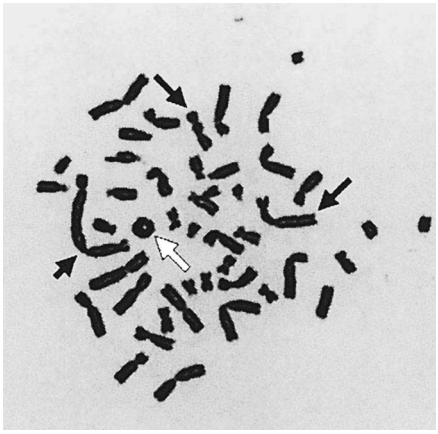

Fig. 3. Metaphase chromosomes having dicentric chromosomes (black arrows), a tricentric chromosome (short arrow) and a ring chromosome with centromere (white arrow) in a lymphocyte of patient B.

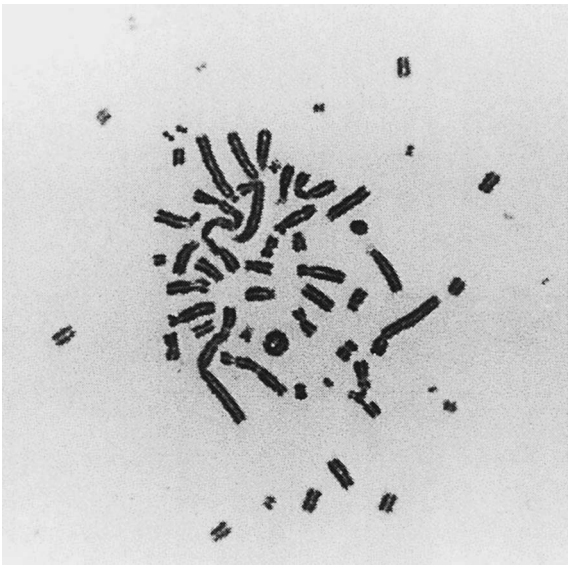

Fig. 4. Metaphase chromosomes having severe chromosome aberrations in a lymphocyte of patient A.

frequencies at three points of time were combined into one for the dose estimation. The estimated doses were 22.6 GyE'' (Gy equivalent to 1.9 MeV X-rays) by Dic and 24.5 GyE'' by Dic+R for patient A, 8.3 GyE'' by Dic as well as by Dic+Rc for patient B (Fig. 5), and 3.0 (2.8–3.2) GyE (Gy equivalent to ^{60}Co γ-rays) by Dic+Rc for patient C, respectively. These estimated doses were in good accordance with those estimated by the PCC-ring analysis. As far as we know, the estimated dose of the most severely exposed patient in the present study is the highest recorded among the doses that chromosome analyses have been able to estimate in humans.

A comparison of the estimated doses with the physically estimated dose is given in Table 2.

Table 1. Frequency of chromosome aberrations in the lymphocytes in 3 patients after the accident.

Patient	Aberration	Frequency of aberration/cell after accident			
		9 hrs	23 hrs	48 hrs	Total
A	Dic	445/50	197/20	73/8	715/78
	Dic+R	563/50	250/20	90/8	903/78
B	Dic	199/75	127/50	153/50	479/175
	Dic+Rc	224/75	147/50	166/50	537/175
C	Dic+Rc	63/100	64/100	64/100	191/300

Dic: dicentric chromosome, R: ring chromosome with/without centromere
Rc: ring chromosome with centromere

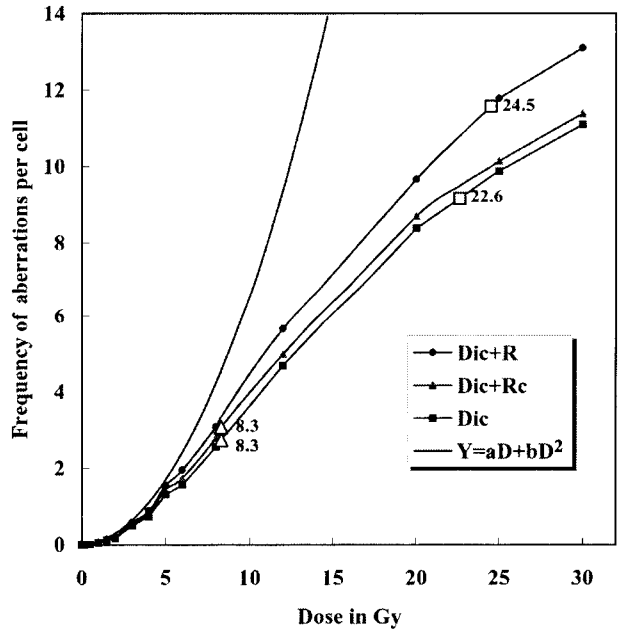

Fig. 5. Dose-response curve ($Y = 2.31 \times 10^{-2}D + 6.33 \times 10^{-2}D^2$) of Dic+Rc for ^{60}Co γ-rays, dose-response curves of Dic+R, Dic+Rc, and Dic for 1.9MeV X-rays (after Norman and Sasaki, 1966)[6], and the estimated doses of patients A(□) and B(△).

A trial calculation the of γ-ray equivalent dose (GyE) based on the physical dose estimated by the measurement of ^{24}Na is reported by Ishigure et al.[7] in this issue. The doses estimated by chromosome analyses were in good agreement with the doses estimated by the ^{24}Na measurement when the neutron's RBE was assumed to be 1.5–2.0.

Stem cells were transplanted in patients A and B. The transplantation was successful in both patients. The transplanted cells were gradually replaced by the host cells in B. Unfortunately,

Table 2. Comparison of the doses estimated by various indicators.

Patient	Estimated Dose (GyE, GyE', or GyE'')* by			
	PCC-Ring	Dic	Dic+R/Rc	^{24}Na**
A	>20	22.6	24.5	17–24
B	7.4(6.5–8.2)	8.3	8.3	8.7–13
C	2.3(1.8–2.8)	–	3.0(2.8–3.2)	2.5–3.6

* Equivalent dose to X or γ-rays ** Ishigure et al.[7], when neutron's RBE is 1.5–2.0.

patient A passed away 83 days after the accident and patient B at 211 days. Patient C recovered and was released from the hospital 3 months after the accident.

ACKNOWLEDGEMENTS

Permission for publishing the results of the clinical and biological examinations of the present 3 patients for academic purposes was given from Mrs. A, patient B and patient C. We could overcome problems concerning the dosimetry of those three patients by cytogenetical techniques which we had developed under the Nuclear Cross Over Research Project supported by the Science and Technology Agency. We thank Ms. Yoshiko Kawase, Ms. Seiko Suzuki, Ms. Yoko Yamagishi and Ms. Yasuko Morimoto for their technical assistance.

REFERENCES

1. Hayata, I., Tabuchi, H., Furukawa, A., Okabe, N., Yamamoto M. and Sato, K. (1992) Robot system for preparing lymphocyte chromosome. J. Radiat. Res. **33**: Suppl. 231–241.
2. Kanda, R., Hayata, I. and Lloyd, D. C. (1999) Easy biodosimetry for high-dose radiation exposures using drug-induced, prematurely condensed chromosomes. Int. J. Radiat. Biol. **75**: 441–446.
3. Hayata, I. (1998) Advanced cytogenetical techniques necessary for the study of low dose exposures. In: High Levels of Natural Radiation: Radiaton Dose and Health Effects. Eds. L-X. Wei, T. Sugahara and Z. Tao, pp. 293–300, Elsevier, Amsterdam.
4. IAEA (1986) Biodosimetry: Chromosomal aberration analysis for dose assessment. IAEA Technical Report Series 260, IAEA, Vienna.
5. Lloyd, D. C. and Edward, A. A. (1983) Chromosome aberrations in human lymphocytes: Effect of radiation quality, dose and dose rate. In: Radiation-induced Chromosome Damage in Man. Eds. by T. Ishihara and M. S. Sasaki, pp. 23–49, Alan R. Liss, Inc., New York.
6. Norman, A. and Sasaki, M. S. (1966) Chromosome-exchange aberrations in human lymphocytes. Int. J. Radiat. Biol. **66**: 321–328.
7. Ishigure, N., Endo, A., Yamaguchi, Y. and Kawachi, K. (2001) Calculation of the absorbed dose for the overexposed patients at the JCO Criticality accident in Tokai-mura. J. Radiat. Res. **42**: Suppl., S137–S148.