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Short communication

Transfusion of γ-irradiated blood components to individuals does not compromise the cytogenetic dose assessment

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ABSTRACT

Most blood components for transfusions are irradiated *ex vivo* to prevent transfusion-associated graft-versus-host disease (TA-GvHD); this irradiation can potentially affect the cytogenetic dose assessment of patients showing acute radiation syndrome (ARS) with bone marrow suppression or acute anaemia. Whole blood samples from five donors were irradiated with 0, 10 or 25 Gy γ-rays. The mitotic activity of each cultured blood sample was measured by calculating the mitotic index. A dicentric chromosome assay was used to evaluate the chromosomal aberrations and absorbed dose of blood lymphocytes. Mitogenic activity and scorable metaphase spreads were significantly decreased in the blood samples irradiated with 10 and 25 Gy ($p < 0.001$). Moreover, a significant increase in the mean scores of all types of chromosomal aberrations in the 10 Gy γ-irradiated samples was observed, with the estimated dose being 11.3 Gy (95% CI: 10.67–11.95 Gy); however, we were unable to estimate the exposure dose in the 25 Gy γ-irradiated samples due to a limited number of scorable metaphase spreads. The mitotic index of the 25 Gy γ-irradiated whole blood samples was significantly suppressed by more than 4-log fold. Thus, in the present study, we evaluated the effects of recommended radiation doses in γ-irradiated transplantation blood components using cytogenetic dosimetry. These results suggest that the partial transfusion of blood components to patients with ARS or acute anaemia did not compromise the estimation of the exposure dose using cytogenetic dosimetry.

1. Introduction

Irradiation of blood and blood components is presently the only accepted methodology to prevent transfusion-associated graft-versus host disease (TA-GvHD), a rare but fatal complication that occurs in susceptible individuals when immunocompetent lymphocytes are transfused into a recipient who is unable to respond to the cells due to one-way human lymphocyte antigen (HLA) compatibility and/or immunosuppression. This results in the engraftment of transfused cells, which then proceeds to reject the host due to immunological differences. In particular, TA-GvHD severely affects immunocompromised patients, such as individuals with congenital immunodeficiency, after bone marrow transplantation, radiotherapy, or chemotherapy. TA-GvHD is routinely prevented by the irradiation of blood components using γ-rays (or X-rays) to inactivate the T lymphocytes [1]. Thus, all blood components that might contain viable T lymphocytes, including whole blood and cellular components, packed red blood cells (RBCs), concentrated platelets, granulocytes, and fresh plasma, should be removed in patients who are at risk of TA-GvHD. Several investigators have reported the recommended gamma- and X-ray doses for inactivating T lymphocytes and have demonstrated that the absorbed dose necessary to prevent TA-GvHD without damaging the granulocytes and anucleate cells, i.e., erythrocytes and platelets, is 25 Gy [2–6]. Moreover, other investigators reported that the minimum possible radiation dose necessary for the efficient inactivation of lymphocytes in blood components and products is 25 Gy [7,8]. Thus, the Food and Drug Administration and the American Association of Blood Banks (AABB) specify the recommended dose of 25 Gy to the mid-plane of the irradiated blood unit and that a minimum dose of 15 Gy should be delivered throughout the unit [9,10]. No significant change was observed in the ultrastructure and function of RBCs upon irradiation with 35 Gy of ^{137}Cs γ-rays [11].

Nevertheless, ionising irradiation (IR) damages the DNA within cells

Abbreviations: ARS, acute radiation syndrome; DSB, double-strand break; IR, ionising irradiation; MI, mitotic index; TA-GvHD, transfusion-associated graft-versus host disease.

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via the production of discrete energy-depositing factors; it induces various direct and indirect damages in DNA such as base damage, single-strand breaks, double-strand breaks (DSBs), and DNA–protein cross-links. Furthermore, DSBs may constitute the lesions that lead to the production of chromosome aberrations [12]. Cytogenetic studies have demonstrated that low levels of chronic exposure may increase the frequencies of chromosomal aberrations. Chromosomal aberrations have been analysed in biological dosimetry studies for over 60 years in order to evaluate the cases of accidental IR over-exposure [13]. The aberrations scored in lymphocytes are interpreted in terms of absorbed dose by reference to a dose-response calibration curve. The biological endpoint of the scored chromosome aberrations reflects the dose to the cell nuclei. The dose value obtained by referring to the measured yield of aberrations, such as dicentric chromosomes, to a calibration curve, represents the average absorbed dose to the lymphocytes. This can be extrapolated to the average whole-body dose because lymphocytes circulate throughout the body.

Patients with acute radiation syndrome (ARS) and bone marrow suppression or acute anaemia frequently require multiple transfusions with various blood products. In these cases, the clinical management is dependent on the absorbed dose measured via cytogenetic dosimetry or other dosimetric methods for the appropriate evaluation of exposure doses. In the present study, we investigated the changes in mitogenic activity and unstable chromosome aberrations in whole blood cultures after irradiation with different doses of ^{137}Cs γ-ray to evaluate the effect of blood transfusion on cytogenetic dosimetry.

2. Materials and methods

2.1. Blood samples

Blood samples were obtained from five healthy donors who were not exposed to IR for occupational purposes after approval from the Institutional Review Board for Research on Human Subjects at Korea Institute of Radiological and Medical Sciences (No. K-1707-001-003). Written informed consent for participation in this study was obtained from each individual.

2.2. Irradiation, blood cell culture, and scoring of chromosomal aberrations

Heparinised whole blood samples were irradiated by a γ-ray blood irradiator (^{137}Cs source; dose rate, 3.25 Gy/min; Gammacell 3000 Elan, Ottawa, Canada) with doses of 10, 25, or 0 Gy (control).

Culturing, harvesting, staining, and scoring were performed according to the technical manual of the Korea Laboratory Accreditation Scheme accreditation developed in our laboratory and in accordance with the International Atomic Energy Agency recommendations with well-trained two scorers [13,14]. Whole blood was cultured for 48 h in RPMI-1640 medium supplemented with 20% (v/v) foetal bovine serum, 2% phyto-haemagglutinin, and antibiotics in a humidified 5% CO_2 incubator at 37 °C. Colcemid (0.07 μg/mL) was added 24 h before harvest. The cells were harvested after swelling in 0.075 M KCl, pre-warmed at 37 °C, and then fixed at least thrice in 3:1 methanol/glacial acetic acid. The slides with well-spread metaphase cells were incubated at 60 °C overnight. For conventional chromosomal aberration analysis, the slides were stained with 4% Giemsa solution in phosphate buffer (pH 6.8) for 10 min.

2.3. Mitotic index (MI) calculation

Mitotic index (MI) was determined by scoring at least 30,000 cells from each dose group and was calculated as the ratio of the number of metaphase cells to the total number of stimulated cells (metaphase cells, *M*; blast cells with large nuclei, *B*), excluding unstimulated cells with small nuclei as well as dead or dying cells (Eq. 1).

$$MI = (M \times 100)/(M + B) \quad (1)$$

2.4. Dose estimation by the scored dicentrics

The absorbed dose for each blood sample was calculated from the measured yield of dicentrics using our own dose-response calibration curves [14]. The equation for dicentrics was as follows: $Y = 0.0011\ (\pm 0.0001) + 0.0357\ (\pm 0.0042)\ D + 0.0642\ (\pm 0.0025)\ D^2$ (Y: yield of dicentrics, D: dose (Gy)). The estimated dose was calculated using DoseEstimate v5.2 (Public Health England, London, England).

2.5. Statistical analysis

All statistical analyses were performed using commercial software (SPSS version 23 Windows version; IBM; Armonk, NY, USA). Differences with p values < 0.001 were considered statistically significant.

3. Results

3.1. Lymphocyte mitosis induced by ex vivo γ-irradiation

The demographic characteristics of the study subjects are presented in Table 1. In the present study, we investigated the MI and the frequencies of chromosomal aberration occurring due to γ-irradiation. We found that the MI significantly decreased ($p < 0.001$) in the γ-irradiated samples (10 Gy γ-irradiated samples, 0.09 ± 0.03; 25 Gy γ-irradiated samples, 0.003 ± 0.002) compared to that in the non-irradiated control samples (15.10 ± 1.59). A low-magnification view of a typical lymphocyte culture is illustrated in Fig. 1A and B. The dotted black boxes indicate the metaphase spreads. The number of metaphase spreads that were detected in the 10 slides prepared from 10 and 25 Gy γ-irradiated samples were 47 and 1, respectively.

3.2. Chromosomal aberrations in lymphocytes subjected to ex vivo γ-irradiation

In total, 5,000 metaphase spreads of the control samples were analysed using the dicentric assay, which revealed almost normal features with 46 centromeres and 46 chromosomes (Fig. 2A). The dicentric chromosome frequency of the non-irradiated control samples was 1.90 ± 0.51 per 1,000 metaphase spreads, which was similar to the normal background level reported in our previous study and studies by other research groups [14]. The scorable major chromosomal aberrations for the γ-irradiated blood groups were multicentrics, centric rings, and excess acentric fragments, as illustrated in Fig. 2B. The mean scores of all types of chromosomal aberrations significantly increased in the 10 Gy γ-irradiated samples, and any one cell included 4–14 chromosomal aberrations (Table 2).

3.3. Dose estimation following high-dose exposure using the dose-response calibration curve

We observed a significant amount of chromosomal aberrations, which comprised 2 tetracentrics, 38 tricentrics, 245 dicentrics, and 31 centric rings, in the 38 scorable metaphase spreads of 10 Gy γ-irradiated whole blood samples. Notably, 9 unscorable metaphase spreads were

Table 1
Study population age. All participants are nonsmokers and have no experience of medical IR exposure except chest X-ray.

Gender	Number of subjects (%)	Years (Mean ± SE, range)
Female	3 (60)	30.3 ± 0.3 (30–31)
Male	2 (40)	32.0 ± 2.0 (30–34)

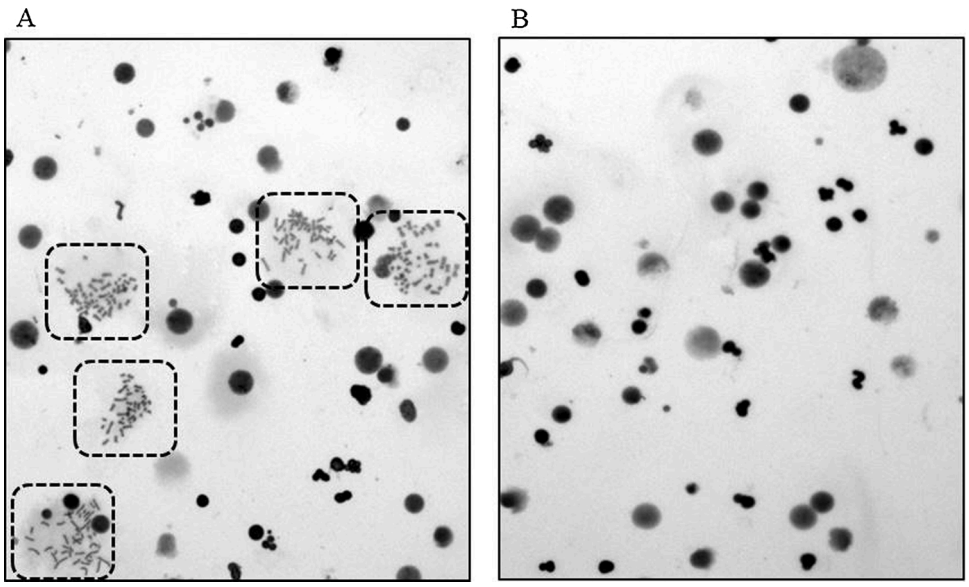

Fig. 1. Comparison of the mitotic index of the control and irradiated samples. Low-magnification view of the lymphocyte culture slides of the (A) non-irradiated control samples and (B) 10 Gy-irradiated samples. The dotted black boxes indicate the metaphase spreads.

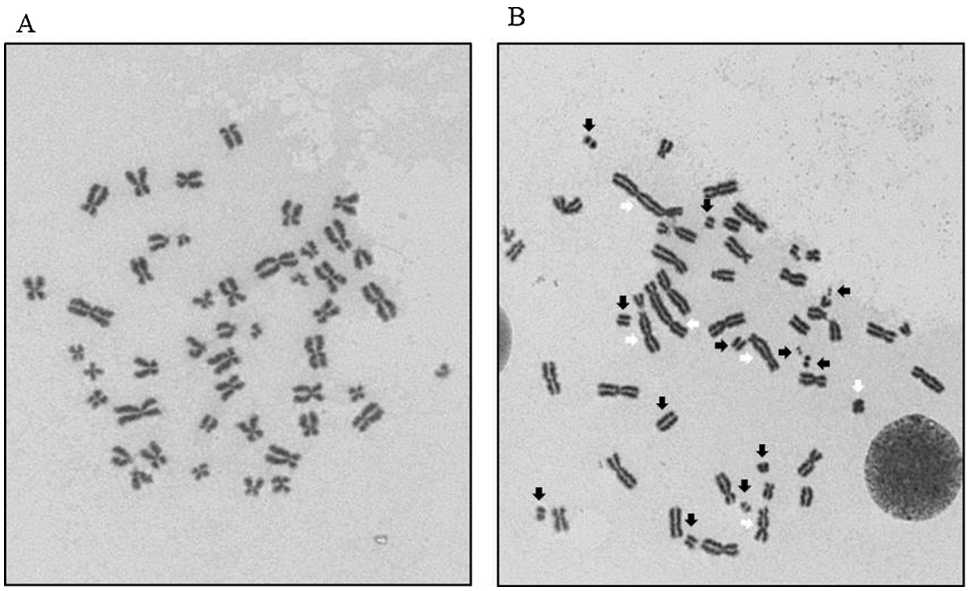

Fig. 2. Comparison of metaphase spreads of the (A) non-irradiated control samples and (B) 10 Gy-irradiated samples. The arrows indicate chromosomal aberrations with dicentrics and centric rings (white) and acentrics with double minutes (black).

Table 2
Mitotic index (MI) was determined by scoring at least 50,000 cells from each dosage group; thereafter, the MI was calculated as the ratio of the number of metaphase cells to the total number of irradiated cells.

Subjects	0 Gy			10 Gy		
	B (*N*)	M (*N*)	MI (%)	B (*N*)	M (*N*)	MI (%)
A	11,320	1,622	12.53	9,789	14	0.14
B	12,360	2,871	18.85	10,250	11	0.11
C	22,910	3,833	14.33	19,175	21	0.11
D	9,361	1,205	11.40	8,791	2	0.02
E	7,251	1,709	19.07	6,358	1	0.02
Total	63,202	11,240	15.10	54,363	47	0.09

B = blast cells with large nuclei; M = metaphase cells; *N* = number of cells.

excluded based on the scoring criteria (Table 3). The estimated dose for the 10 Gy γ-irradiated samples using our own dose-response calibration curve was 11.3 Gy (95% CI: 10.67–11.95 Gy); however, we were unable to estimate the exposure dose for the 25 Gy γ-irradiated samples because the number of scorable metaphase spreads was insufficient.

4. Discussion

White blood cells (WBCs) in transfused blood components can cause clinical problems, despite their crucial roles in immunology and *in vivo* disease protection. The rationale for preventing WBC-mediated adverse effects suggests leukocyte reduction as an attractive approach. The use of leukocyte reduction to prevent TA-GvHD, which is caused by the presence of viable lymphocytes in blood, remains speculative and unknown [15]. Blood component filtration often reduced the counts of WBCs by more than 5-log fold [16].

Table 3
Dose estimation of the whole blood samples irradiated with 10 Gy γ-rays using the KIRAMS dose-response calibration curve (Source: ^{60}Co, Dose rate =0.5 Gy/min). The estimated dose was calculated using DoseEstimate v5.2.

Subjects	Scored M cells	Multicentrics			Rings	Excess acentric frgs	Yield of Dic	Dispersion index	U value	Estimated dose (Gy)	95% CI	
		Tetrac	Tric	Dic							LDL (Gy)	UDL (Gy)
A	12	1	11	74	8	31	8.25	0.95	−0.12	11.06	9.95	12.23
B	9	0	10	64	8	8	9.33	0.80	−0.40	11.78	10.49	13.14
C	15	1	13	97	14	25	8.40	0.81	−0.50	11.16	10.16	12.21
D	1	0	2	4	1	0	8.00	–	–	–	–	–
E	1	0	2	6	0	0	10.00	–	–	–	–	–
Total	38	2	38	245	31	64	8.61	0.79	−0.91	11.30	10.67	11.95

Multicentrics = (N - 1) dicentrics; M = metaphase; Tetrac = tetracentrics; Tric = tricentrics; Dic = dicentrics; frgs = fragments; CI = confidence interval; LDL = low dose limit; UDL = upper dose limit.

Blood irradiation is majorly used to prevent TA-GvHD in immunodeficient patients via the elimination of T lymphocytes. Lymphocytes are one of the most radio-sensitive mammalian cells [17]. Although irradiation alters neither lymphocyte count nor their viability, Wong et al. reported an impairment in their function following treatment with radiation dose of 30 Gy [18]. Pelzinsky et al. reported that a minimum radiation dose of 25 Gy is required to effectively inactivate lymphocytes in the RBC units [5].

To evaluate the MI for dicentric assay, (a) the nuclei were excluded from polymorphonuclear cells, unstimulated cells (small nuclei), dead or dying cells, and micronuclei; (b) the number of nuclei from mitotic cells and stimulated cells (blast cells with large nuclei) was determined; Eq. 1 was used to calculate the MI of stimulated cells (Fig. 1B–D). Our data confirmed that the mitogenic activity dramatically decreased in blood lymphocytes after treatment with ^{137}Cs γ-irradiation, and even 10 Gy γ-irradiation reduced the MI by 2.2-log fold. The blood irradiation dose recommended by the Transfusion Guideline in South Korea (4th edition), 25 Gy γ-irradiation, reduced the MI by 3.7-log fold [19]. The AABB, Korea Center for Disease Control (KCDC), and Japanese Society of Blood Transfusion (JSBT) recommend a γ-irradiation dosage of 25 Gy for the central portion of the blood components, with no portion of the bag receiving less than 15 Gy γ-irradiation [10,19–21].

An enormous amount of chromosomal aberrations was produced in 38 scorable metaphase spreads obtained from 10 Gy γ-irradiated whole blood samples from 5 individuals. Moreover, a unique multi-aberrant cell with a high multicentric count and/or centric rings as well as excess acentrics with double minutes appearing as a rogue cell-like metaphase form appeared in the 10 Gy γ-irradiated samples (subject A and C). The absorbed dose for each blood sample was calculated from the measured yield of dicentrics using our own dose-response calibration curve (Table 3). Our estimated dose in the 10 Gy γ-irradiated samples was assumed to be 11.3 Gy (95% CI: 10.67–11.95), which was slightly higher than the actual dose for the 38 scorable metaphase spreads. This result was obtained by our own dose-response calibration curve generated by GammaBeam (Best Theratronics, Canada) after irradiation with a ^{60}Co source at a dose rate of 0.5 Gy/min. However, this result indicated that this cytogenetic dosimetry system may be applied for estimating the exposure dose for samples subjected to radiation doses of up to 10 Gy.

5. Conclusions

In this study, we examined the impact of the recommended radiation exposure dose to evaluate the effects of γ-irradiated blood components on cytogenetic dosimetry. Mitogenic activity and scorable metaphase spreads significantly decreased in 10 and 25 Gy γ-irradiated blood samples, respectively. The incidence of all types of chromosomal aberrations were significantly increased in 10 Gy γ-irradiated samples. Moreover, the MIs of 25 Gy γ-irradiated samples were significantly suppressed and reduced by more than 4-log fold; therefore, the exposure dose could not be estimated via cytogenetic dosimetry due to the lack of scorable metaphase spreads. Our findings suggest that the partial transfusion of blood components to patients with ARS or acute anaemia did not compromise the estimation of exposure dose using cytogenetic dosimetry.

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IRB statement

This study was approved by the institutional review board of Korea Institute of Radiological and Medical Sciences (No. K-1707-001-003). The subjects enrolled in this study provided written informed consent.

CRediT authorship contribution statement

Seongjae Jang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Sanghyeok Woo:** Methodology, Validation, Investigation. **Yang Hee Lee:** Methodology, Investigation. **Susan Yang:** Methodology, Investigation. **Young Woo Jin:** Writing - review & editing.

Declaration of Competing Interest

The authors declared no potential conflicts of interests with respect to the research, authorship, and/or publication of this article.

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