Biodosimetry Based on Gamma-H2AX Quantification in Human Peripheral Blood Lymphocytes after Partial-body Irradiation

Li-Ping Ma,* Jie Chen,* Meng-Meng Liu, Juan Yan, Jia-Qi Xiang, Mei Tian, Ling Gao, and Qing-Jie Liu[1]

Abstract—**Quantification of gamma-H2AX foci can estimate exposure to ionizing radiation. Most nuclear and radiation accidents are partial-body irradiation, and the doses estimated using the total-body irradiation dose estimation formula are often lower than the actual dose. To evaluate the dose-response relation of gamma-H2AX foci in human peripheral blood lymphocytes after partial-body irradiation and establish a simple and high throughput model to estimate partial-body irradiation dose, we collected human peripheral blood and irradiated with 0-, 0.5-, 1-, 2-, 3-, 4-, 5-, 6-, and 8-Gy gamma rays to simulate total-body irradiation in vitro. Gamma-H2AX foci were quantitated by flow cytometry at 1 h after irradiation, and a dose-response curve was established for total-body irradiation dose estimation. Then, a partial-body irradiation dose-response calibration curve was established by adding calibration coefficients based on the Dolphin method. To reflect the data distribution of all doses more realistically, the partial-body irradiation dose-response calibration curve was divided into two sections. In addition, partial-body irradiation was simulated in vitro, and the PBI data were substituted into curves to verify the accuracy of the two partial-body irradiation calibration curves. Results showed that the dose estimation variations were all less than 30% except the 25% partial-body irradiation group at 1 Gy, and the partial-body irradiation calibration dose-response curves were $YF_1 = -3.444\ x^2 + 18.532\ x + 3.109$, $R^2 = 0.92$ ($YF \leq 27.95$); $YF_2 = -2.704\ x^2 + 37.97\ x - 56.45$, $R^2 = 0.86$ ($YF > 27.95$). Results also suggested that the partial-body irradiation dose-response calibration curve based on the gamma-H2AX foci quantification in human peripheral blood lymphocytes is a simple and high throughput model to assess partial-body irradiation dose.**

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Key words: dosimetry; gamma radiation; radiobiology; modeling, dose assessment

INTRODUCTION

HUMANS ARE exposed to ionizing radiation during various radiological diagnostic and therapeutic or radiation accidents (Sproull et al. 2017; Obrador et al. 2022; Rasmussen et al. 2022). During radiological accidents, determining individual exposure is critical to early emergency triage and provides the best medical procedure for each individual (Flood et al. 2011). However, most of the existing radiation accidents are partial-body irradiation (PBI), and the biological dosimeters are mostly for total-body irradiation (TBI), which is not accurate for PBI. In other words, the PBI dose evaluated by the dose-response curve of TBI is often lower than the actual exposure dose. At present, the use of dicentric chromosome aberration (DCA) assay to assess PBI dose has many limits when applied because it requires a long cell culture time and high technical requirements for operators, so there is an urgent need to develop a biodosimeter to evaluate the dose of PBI rapidly.

The DNA double-strand breaks (DSBs) caused by ionizing radiation have the characteristic of increasing the number of broken strands with irradiation dose. H2AX molecules are immediately phosphorylated at serine 139 (gamma-H2AX) after the formation of DSBs (Nakamura et al. 2010; Collins et al. 2020). Staining gamma-H2AX foci with gamma-H2AX antibody and counting gamma-H2AX foci in many tissues, including lymphocytes, hair follicles, and skin biopsies is the most sensitive method for quantitative evaluation of DSBs and irradiation doses (Wang et al. 2014). To investigate the potential of using gamma-H2AX as a biodosimeter at an early stage, a rapid and high-throughput approach was established for detecting the levels of gamma-H2AX in lymphocytes by flow cytometry (Redon et al. 2010, 2011; Johansson et al. 2017; Lee et al. 2019).

For PBI, blood circulation makes a mixture of the non-irradiation and irradiation sections. This mixture leads to changes in gamma-H2AX that are different from those observed during TBI (Kozubek et al. 2001; Vasireddy et al. 2010). The PBI dose assessed by the gamma-H2AX dose response of TBI will be lower than the actual irradiation

[1]China CDC Key Laboratory of Radiological Protection and Nuclear Emergency, National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing 100088, P.R. China.

*These authors contributed equally to this work. The authors declare no conflicts of interest.

For correspondence contact: Ling Gao, China CDC Key Laboratory of Radiological Protection and Nuclear Emergency, National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China, or email at **gaoling@nirp.chinacdc.cn**; Qing-Jie Liu, China CDC Key Laboratory of Radiological Protection and Nuclear Emergency, National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China, or email at **liuqingjie@nirp.chinacdc.cn**.

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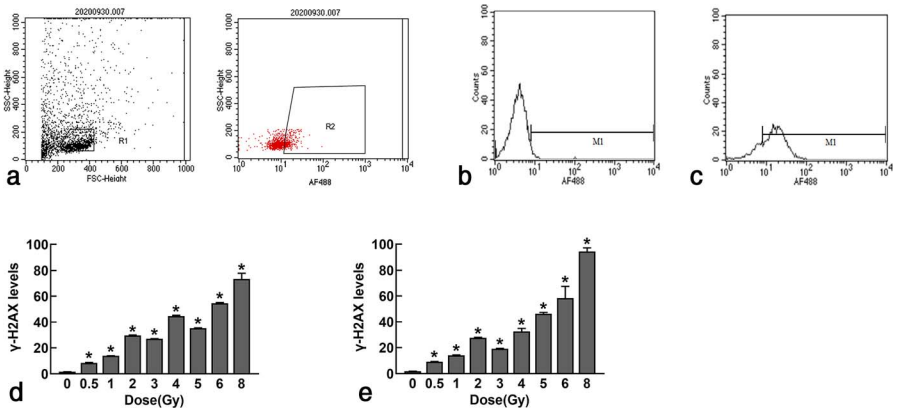

Fig. 1. Quantification of gamma-H2AX foci was analyzed by flow cytometry at 1 h after 0-8 Gy ^{60}Co gamma-ray irradiation. a: Gating on the human peripheral blood lymphocytes based on Forward Scatter (FSC) and Side Scatter (SSC), which was used for all subsequent analyses, and representative diagram of gamma-H2AX sorting according to FSC and Alexa Fluor 488 (AF488). b, c: Gamma-H2AX histograms in lymphocytes at 1 h after 0 Gy (b) or 8 Gy (c) gamma-ray irradiation. d, e: Gamma-H2AX levels in lymphocytes collected from two persons' peripheral blood irradiated in vitro at 1 h after 0, 0.5, 1, 2, 3, 4, 5, 6, and 8 Gy of gamma-ray irradiation. (Error bars represent SD; * means significant difference compared with the 0 Gy group, $P < 0.05$; n = 3.)

dose. Therefore, the establishment of the gamma-H2AX dose-response curve in PBI should be considered.

The dose-response curves based on gamma-H2AX foci were established after 0-8 Gy gamma-rays PBI (^{60}Co gamma rays at 1 Gy min^{-1}) in normal blood samples, which is based on the Dolphin method in IAEA Part 9.7 Dose Assessment (IAEA 2011), by calculating the number of gamma-H2AX foci per damaged cell. Gamma-H2AX foci were confined for lymphocytes because lymphocytes generated a robust gamma-H2AX response to these doses of ionizing radiation.

MATERIALS AND METHODS

Whole-blood sample collection and irradiation

After obtaining written informed consent from volunteers and ethical approval from the Subcommittee on Human Investigation of the National Institute for Radiological Protection (NIRP, act no. LLSC2020-008) under the Chinese Center for Disease Control and Prevention (China CDC), peripheral blood samples from three healthy donors (20-35 y old, who had not been irradiated within 6 mo) were collected using heparinized tubes. The blood sample of each person was equally divided into 0.5-mL aliquots, and then it was irradiated in vitro with 0, 0.5, 1, 2, 3, 4, 5, 6, and 8 Gy ^{60}Co gamma rays at a dose rate of 1 Gy min^{-1} (Beijing Radiation Center). Blood samples were irradiated at different doses by controlling the dose rate and adjusting the irradiation time. Radiation was performed at room temperature. After irradiation, cells were kept on ice during transportation to the laboratory. About 1 h after irradiation is the best time to determine the maximum yield of the gamma-H2AX foci (Chaurasia et al. 2021). Therefore, we incubated blood samples at 37 °C for 1 h after irradiation to detect gamma-H2AX fluorescence intensity using flow cytometry.

Gamma-H2AX detection and quantification in human peripheral blood lymphocytes

Precooled red blood cell lysis buffer (1-mL blood sample mixed with red blood cell lysis buffer at a ratio of 1:10) was added to the whole blood sample, lysed in the dark for about 8 min, and centrifuged to remove the supernatant (250 g, 4 °C, 3 min). Samples were resuspended and washed with cold phosphate-buffered saline (PBS) and centrifuged to remove the supernatant (250 g, 4 °C, 3 min) until the red blood cells were removed. The remaining white blood cells were then collected and fixed in 2% paraformaldehyde (PFA) (1-mL blood sample mixed with PFA at a ratio of

Dose (Gy)	n	Mean ± SEM	F	P
0	3	1.45 ± 0.30		
0.5	3	8.66 ± 0.38		
1	3	13.27 ± 0.62		
2	3	27.05 ± 1.67		
3	3	23.50 ± 2.32	22.116	<0.01
4	3	44.97 ± 7.28		
5	3	29.60 ± 11.57		
6	3	58.57 ± 2.31		
8	3	79.44 ± 7.50		

a

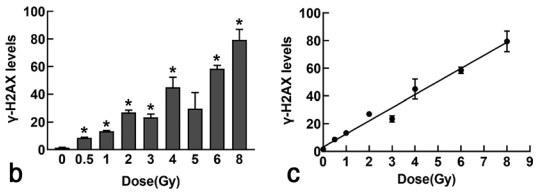

Fig. 2. Dose-response curve elaboration and dose assessment after 0-8 Gy ^{60}Co gamma-ray irradiation. a, b: The average gamma-H2AX levels in lymphocytes of three person's peripheral blood at 1 h after different irradiation doses. c: Dose-response curve for total-body irradiation dose assessment. (Error bar represents SEM; * means significant difference compared with the 0 Gy group, $P < 0.05$; n = 3.)

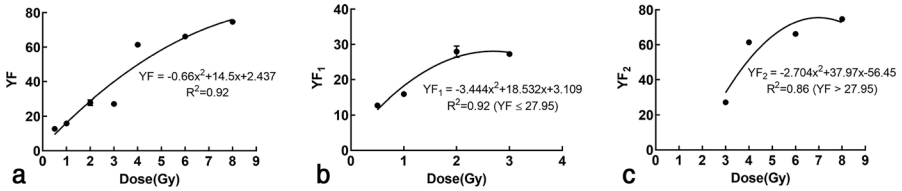

Fig. 3. Fitted dose-response curves between the irradiation dose and $YF_{\gamma\text{-H2AX}}$ to assess partial-body irradiation dose after 0-8 Gy ^{60}Co gamma-ray irradiation. a: The standard dose-response calibration curve for PBI dose assessment was established by adding correction coefficients into the TBI standard dose-response curve. b, c: The standard PBI dose-response curve was divided into two sections. YF refers to the mean fluorescence intensity of gamma-H2AX foci per cell in the cells containing foci, ignoring the foci-free cells, and x means the dose of irradiation.

1:2) for 30 min at room temperature. PFA was removed by centrifugation (250 g, 4 °C, 3 min) and washed once with PBS. Samples were resuspended with PBS followed by the addition of precooled 100% methanol and permeabilized on ice for 10 min, then centrifuged (500 g, 4 °C, 5 min) to remove methanol and washed once with PBS. The samples were stained with Alexa Fluor 488 (AF488)-conjugated rabbit anti-phospho-histone H2A.X (Ser139) (20E3) mAb (Cell Signaling Technology, Danvers, MA; 1:50 dilution) solution and incubated at room temperature in the dark for 1 h. Finally, the samples were washed twice with cold PBS (500 g, 4 °C, 3 min), and resuspended with 200 μL PBS. The prepared samples were tested by flow cytometry.

We used a flow cytometer (BD FACS Calibur) to select lymphocyte populations by cell size based on forward scatter (FSC) and side scatter (SSC), which ensured that approximately 10,000 lymphocytes were analyzed per experiment. The flow cytometer had a laser wavelength of 488 nm, with laser powers of 15 mW, and a gain of 1. The fluorescence intensity values of gamma-H2AX in total lymphocytes labeled with immunofluorescence anti-gamma-H2AX-antibody were analyzed by flow cytometry.

In addition, the blood samples irradiated with different doses were mixed with unirradiated blood samples at the proportions of 25%, 50%, and 75% to simulate PBI. The values of gamma-H2AX in lymphocytes were analyzed by flow cytometry at 1 h after irradiation.

PBI calculations based on the Dolphin method

This method is based on the Dolphin method (formula 13) in Part 9.7 Dose Assessment of IAEA (2011). The term $YF_{\gamma\text{-H2AX}}$, referring to the mean fluorescence intensity of gamma-H2AX foci per cell in the cells containing foci and ignoring the foci-free cells, which is a correction for Y. $YF_{\gamma\text{-H2AX}}$, can be calculated with the following formula (the deformation of the Dolphin method):

$$YF_{\gamma\text{-H2AX}} = X_{\gamma\text{-H2AX}} / \left(N \times F_{\gamma\text{-H2AX}}\right) \quad (1)$$

where N is the total number of lymphocytes analyzed, $X_{\gamma\text{-H2AX}}$ is gamma-H2AX fluorescence intensity of total lymphocytes, and $F_{\gamma\text{-H2AX}}$ is the fraction of the lymphocyte population containing gamma-H2AX foci. $F_{\gamma\text{-H2AX}}$ could be obtained by flow cytometry directly.

We determined the relationship between $YF_{\gamma\text{-H2AX}}$ and doses based on TBI data. The relationship between $F_{\gamma\text{-H2AX}}$ and doses was also established by TBI date. Then, the dose-response calibration curves were constructed to estimate the irradiation dose and area fraction exposed in PBI incidents.

To estimate the area fraction exposed to PBI, the measured values $YF_{\gamma\text{-H2AX}}$ and $F_{\gamma\text{-H2AX}}$ were obtained by blood samples after discovering PBI. Use the $YF_{\gamma\text{-H2AX}}$ calibration curves to estimate the dose corresponding to $YF_{\gamma\text{-H2AX}}$, and substitute it into the $F_{\gamma\text{-H2AX}}$ calibration curve to estimate the $F'_{\gamma\text{-H2AX}}$ value under TBI. The $F'_{\gamma\text{-H2AX}}$ value calculated using TBI curves is much higher than the measured $F_{\gamma\text{-H2AX}}$ value, and the difference between $F'_{\gamma\text{-H2AX}}$ and $F_{\gamma\text{-H2AX}}$ values would indicate the occurrence of PBI. PBI area fraction can be calculated by the ratio after subtraction of the background value:

Table 1. Dose estimation results of simulated partial-body irradiation on human peripheral blood in vitro ($YF = -0.66\,x^2 + 14.5\,x + 2.437$, $R^2 = 0.92$).

Simulated PBI (Dose, the fraction of partial-body exposures)	$YF_{\gamma\text{-H2AX}}$	Estimated dose (Gy)	Variation (%)
1 Gy, 25%	6.64	0.294	70.60
1 Gy, 50%	14.41	0.859	14.10
1 Gy, 75%	18.10	1.139	13.90
5 Gy, 25%	50.68	4.088	18.20
5 Gy, 50%	46.98	3.693	26.10
5 Gy, 75%	46.19	3.611	27.80

Table 2. When YF ≤ 27.95, the dose estimation results of simulated partial-body irradiation on human peripheral blood *in vitro* ($YF_1 = -3.444\ x^2 + 18.532\ x + 3.109$, $R^2 = 0.92$).

Simulated PBI (Dose, the fraction of partial-body exposures)	$YF_{\gamma\text{-H2AX}}$	Estimated dose (Gy)	Variation (%)
1 Gy, 25%	6.64	0.198	80.20
1 Gy, 50%	14.41	0.701	29.90
1 Gy, 75%	18.10	0.992	0.80

$$\text{PBI area fraction} = \frac{\text{Measured } F_{\gamma-H2AX} - \text{background}}{F'_{\gamma-H2AX} \text{ under TBI} - \text{background}} \times 100\%. \quad (2)$$

Measured $F_{\gamma\text{-H2AX}}$ was determined by flow cytometry, and $F'_{\gamma\text{-H2AX}}$ under TBI was determined by the $F_{\gamma\text{-H2AX}}$ calibration curves. The background value for sham irradiated lymphocytes in this study was 0.12.

Statistical analyses

All experiments were repeated three times, and data were expressed as mean ± standard deviation (SD) unless mentioned otherwise. Statistical differences of the gamma-H2AX levels in lymphocytes exposed to all doses compared to those in the non-irradiated group from the same donor were determined by t-test. A difference with $P < 0.05$ was considered statistically significant.

RESULTS

Gamma-H2AX detection and quantification in human peripheral blood lymphocytes after ^{60}Co gamma-ray irradiation in vitro

To assess the feasibility of gamma-H2AX foci fluorescence-based quantitation as a radiation exposure biodosimeter, gamma-H2AX foci in human peripheral blood lymphocytes were detected and quantified by flow cytometry at 1 h after ^{60}Co gamma rays TBI in vitro (Fig. 1a). The gamma-H2AX level was increased in histograms of lymphocytes after 8-Gy gamma-ray irradiation compared with the 0 Gy group ($P < 0.05$) (Fig. 1b and c). Three independent experiments were performed using blood samples from peripheral blood in three different persons that were irradiated in vitro with 0, 0.5, 1, 2, 3, 4, 5, 6, and 8 Gy of gamma rays. Results showed that the level of gamma-H2AX in lymphocytes from different persons overall increased in a dose-dependent manner at 1 h after irradiation ($P < 0.05$) (Fig. 1d and e). Thus, human peripheral blood lymphocytes generated a robust gamma-H2AX response to different irradiation doses.

Human peripheral blood lymphocytes generated a robust gamma-H2AX dose response after total-body ^{60}Co gamma-ray irradiation

To explore the dose-response relationship between the levels of gamma-H2AX in lymphocytes and radiation doses at 1 h after irradiation, the average changes of gamma-H2AX levels were evaluated in lymphocytes at 1 h after different irradiation doses. Fig. 2a shows the data from the three volunteers in three independent experiments. The levels of gamma-H2AX in lymphocytes exposed to all doses indicated were higher than those of the non-irradiated group ($P < 0.05$) (Fig. 2b). Moreover, a dose-response curve was constructed to determine the relationship between the levels of gamma-H2AX and irradiation doses (0-8 Gy) (Fig. 2c). Therefore, the quantitation of gamma-H2AX level in lymphocytes detected at 1 h after irradiation by flow cytometry could be used as an internal biodosimeter for TBI dose assessment.

Dose-response calibration curve for PBI dose assessment

A partial-body exposure analysis method was introduced, $YF_{\gamma\text{-H2AX}}$, which refers to the mean fluorescence intensity of gamma-H2AX foci per cell in the cells containing foci. The $YF_{\gamma\text{-H2AX}}$ dose-response calibration curve was established to assess PBI by correcting the results of peripheral blood lymphocytes after 0-8 Gy TBI using the Dolphin method. The $YF_{\gamma\text{-H2AX}}$ dose-response calibration curve is $YF = -0.66\ x^2 + 14.5\ x + 2.437$ ($R^2 = 0.92$) (Fig. 3a). Data from peripheral blood lymphocytes irradiated with simulated PBI were substituted into the $YF_{\gamma\text{-H2AX}}$ calibration curve, and the variations were less than 30% except for the 1 Gy 25% PBI group (Table 1).

Table 3. When YF > 27.95, the dose estimation results of simulated partial-body irradiation on human peripheral blood *in vitro* ($YF_2 = -2.704\ x^2 + 37.97\ x - 56.45$, $R^2 = 0.86$).

Simulated PBI (Dose, the fraction of partial-body exposures)	$YF_{\gamma\text{-H2AX}}$	Estimated dose (Gy)	Variation (%)
5 Gy, 25%	50.68	3.910	21.79
5 Gy, 50%	46.98	3.698	26.05
5 Gy, 75%	46.19	3.654	26.92

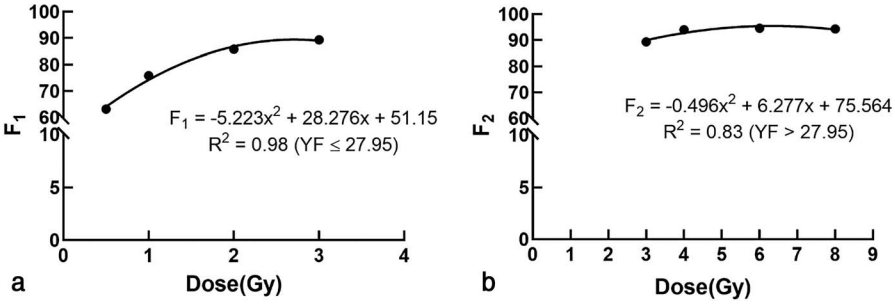

Fig. 4. Fitted dose-response curves between the irradiation dose and $F_{\gamma\text{-H2AX}}$ to assess partial-body irradiation area fraction after 0-8 Gy ^{60}Co gamma-ray irradiation. a, b: The standard PBI dose-response curve was divided into two sections. F refers to the fraction of the lymphocyte population containing gamma-H2AX foci, and x means the dose of irradiation.

To reflect the data distribution of all doses more realistically, the PBI dose-response calibration curve was divided into two sections: if the YF ≤ 27.95, $YF_1 = -3.444\ x^2 + 18.532\ x + 3.109$ ($R^2 = 0.92$) (Fig. 3b), and if the YF > 27.95, $YF_2 = -2.704\ x^2 + 37.97\ x - 56.45$ ($R^2 = 0.86$) (Fig. 3c). After the data of the peripheral blood lymphocytes irradiated with simulated PBI were substituted into the two separate calibration curves, the variations of different PBI fractions were all less than 30% except for the 1 Gy 25% PBI group (Tables 2 and 3).

Dose-response calibration curve for PBI area fraction estimation

A partial-body exposure analysis method was introduced, $F_{\gamma\text{-H2AX}}$, which refers to the proportion of the lymphocyte population containing gamma-H2AX foci. The $F_{\gamma\text{-H2AX}}$ dose-response calibration curve was established using the results of peripheral blood lymphocytes after total-body exposures to 0-8 Gy gamma-rays TBI. Here, the dose-response calibration curve was divided into two sections: if the YF ≤ 27.95, $F_1 = -5.223\ x^2 + 28.276\ x + 51.15$ ($R^2 = 0.98$) (Fig. 4a); and if the YF > 27.95, $F_2 = -0.496\ x^2 + 6.277\ x + 75.564$ ($R^2 = 0.83$) (Fig. 4b). Use the $YF_{\gamma\text{-H2AX}}$ calibration curves in Fig. 3 to estimate the dose corresponding to $YF_{\gamma\text{-H2AX}}$ and substitute it into the $F_{\gamma\text{-H2AX}}$ calibration curve in Fig. 4 to estimate the $F'_{\gamma\text{-H2AX}}$ value under TBI. $F_{\gamma\text{-H2AX}}$ obtained by irradiating peripheral blood lymphocytes with simulated PBI, with proportions of 25%, 50%, and 75%, was substituted into the PBI area fraction formula for assessing the acute area fraction of PBI. The data showed that the variations in estimating the proportion of irradiated area were less than 10% when the actual irradiation dose was 1 Gy (Table 4); The variations in estimating the proportion of irradiated area was less than 30% when the actual irradiation dose was 5 Gy (Table 5).

Table 4. When YF ≤ 27.95, the area fraction estimation results of simulated partial-body irradiation on human peripheral blood in vitro ($F_1 = -5.223\ x^2 + 28.276\ x + 51.15$, $R^2 = 0.98$).

Simulated PBI (Dose, the fraction of partial-body exposures)	Estimated dose (Gy)	$F'_{\gamma\text{-H2AX}}$	Area fraction (%)	Variation (%)
1 Gy, 25%	0.198	56.54	22.76	2.24
1 Gy, 50%	0.701	68.41	57.55	7.55
1 Gy, 75%	0.992	74.05	80.42	5.42

Comparison of gamma-H2AX and Dicentric Chromosome Aberration (DCA)

Blinded samples to the same dose (1, 5 Gy) were analyzed using the gold standard DCA Assay. According to the calibration dose-response curve of DCA established by our group: $y = 5.89 \times 10^{-4} + 1.45 \times 10^{-2}\ D + 5.41 \times 10^{-2}\ D^2$, estimates the corresponding dose. This calibration curve uses ^{60}Co gamma rays at a dose rate of 0.278 Gy min^{-1} and was constructed with doses ranging from 0-6 Gy. Four hundred to 1,000 metaphases were analyzed for each dose point (the number of volunteers was 2, named sample A and sample B). Except for the estimated dose in the 1 Gy 25% PBI group for sample A, which deviated severely from the actual irradiation dose, the relative deviation of the estimated sample irradiated dose from the actual irradiated dose did not exceed 30% in each of the two samples (Tian et al. 2021).

The comparative results are shown in Table 6. For 1 Gy, 25%, the closest estimated dose was 1.11 Gy using DCA, a difference of 11%. For 1 Gy, 75%, the closest dose estimate, 0.992 Gy, was obtained by flow cytometric analysis of gamma-H2AX. There was only a 0.8% difference between the actual and estimated doses. For 5 Gy samples, the estimated dose difference between gamma-H2AX and DCA was within 27%. In conclusion, gamma-H2AX foci analysis gave the closest estimated dose at 1 Gy, 75%.

DISCUSSION

Radiation accidents are more commonly caused by partial-body or inhomogeneous irradiation. Therefore, a PBI biodosimeter of accurate, rapid, and high throughput is important. A PBI analysis method (i.e., $YF_{\gamma\text{-H2AX}}$, $F_{\gamma\text{-H2AX}}$) was introduced. Compared with DCA, assessment using gamma-H2AX foci has the advantage of not requiring the time of cell culture and particularly specialized

Table 5. When YF > 27.95, the area fraction estimation results of simulated partial-body irradiation on human peripheral blood *in vitro* ($F_2 = -0.496 \text{ x}^2 + 6.277 \text{ x} + 75.564$, $R^2 = 0.83$).

Simulated PBI (Dose, the fraction of partial-body exposures)	Estimated dose (Gy)	$F'_{\gamma\text{-H2AX}}$	Area fraction (%)	Variation (%)
5 Gy, 25%	3.910	92.53	41.27	16.27
5 Gy, 50%	3.698	91.99	79.34	29.34
5 Gy, 75%	3.654	91.88	96.78	21.78

techniques, and it is simple and fast to detect the fluorescence intensity of gamma-H2AX and the percentage of gamma-H2AX positive cells by flow cytometry. In this study, we examined and quantified gamma-H2AX produced after ^{60}Co gamma-ray irradiation in vitro of human peripheral lymphocytes. Studies have shown that a dose-response curve based on gamma-H2AX level can be established after ^{60}Co gamma-ray irradiation of human peripheral lymphocytes. The quantification of gamma-H2AX fluorescence intensity level in lymphocytes detected by flow cytometry at 1 h after irradiation can be used as a biodosimeter for assessing TBI dose. Based on the Dolphin method, the $YF_{\gamma\text{-H2AX}}$ dose-response calibration curve and $F_{\gamma\text{-H2AX}}$ dose-response calibration curve were established of peripheral blood lymphocytes after 0- to 8-Gy ^{60}Co gamma rays TBI. The rapid PBI dose and area fraction were evaluated by adding correction coefficients on the TBI dose-response calibration curve.

Redon et al. (2010, 2011) showed that quantifying gamma-H2AX can provide a reliable biodosimeter for the analysis of TBI and PBI in humans. A study by Wang et al. (2014) also used flow cytometry to examine gamma-H2AX protein levels and established the associated dose-response curves, the study by Chaurasia et al. (2021) used both fluorescence microscopy and flow cytometry to quantify the number of gamma-H2AX foci or the percentage of gamma-H2AX positive cells to establish a TBI dose-response calibration curve. However, these studies do not consider the PBI and do not establish a dose-response curve for PBI dose estimation. The study of Horn et al. (2011) pointed out that gamma-H2AX analysis of irradiated lymphocytes allows for a rapid and accurate assessment of TBI doses, and dispersion analysis of foci or intensity distributions helps to determine PBI doses and irradiated area fraction. However, the irradiation dose was limited to the range of 0-4 Gy, and no clear partial-body irradiation dose-response curve was established. Here, a PBI dose-response calibration curve was established using a dose range of 0-8 Gy.

CONCLUSION

For PBI, lymphocyte numbers at the irradiated range and the non-irradiated range were mixed due to blood circulation, and gamma-H2AX levels will be changed. A dose-response model based on TBI is used to evaluate the PBI dose, which will generate a large variation. Therefore, the Dolphin method was used to correct the gamma-H2AX fluorescence intensity and the percentage of positive cells. The segmented simulation based on the corrected gamma-H2AX fluorescence intensity reduces the variation to some extent. The validation data showed that the variation of different PBI groups was less than 30% except for the 1 Gy, 25% PBI group. This is because the partial-body irradiation curves are based on total-body irradiation, and with the increase of the proportion of irradiated area, the partial-body irradiation is close to the total-body irradiation. Therefore, at a lower proportion of irradiated area, there is a greater the deviation from actual dose value. This study is an in vitro experiment with human peripheral blood lymphocytes, and the results could be verified with in vivo samples. However, approximately 1 h after irradiation is the appropriate time to quantify gamma-H2AX foci, and blood samples need to be quickly collected and processed to obtain data after PBI. Further research is needed at more time points to establish models that can be used for PBI dose estimation.

Table 6. Estimation of dose after 1 Gy or 5 Gy ^{60}Co gamma-ray irradiation by gamma-H2AX and DCA assays.

Simulated PBI (Dose, the fraction of partial-body exposures)	Dose estimation by gamma-H2AX assay		Dose estimation by DCA assays			
			Sample A		Sample B	
	Estimated dose (Gy)	Variation (%)	Estimated dose (Gy)	Variation (%)	Estimated dose (Gy)	Variation (%)
1 Gy, 25%	0.198	80.20	2.28	128.00	1.11	11.00
1 Gy, 75%	0.992	0.80	1.15	15.00	1.23	23.00
5 Gy, 25%	3.910	21.79	6.32	26.40	5.87	17.40
5 Gy, 75%	3.654	26.92	5.60	12.00	5.82	16.40

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