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Research article

Comparison of biological measurement and physical estimates of space radiation in the International Space Station

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ABSTRACT

Nowadays, ordinary people can travel in space, and the possibility of extended durations in an environment such as moon of the Earth and Mars with higher space radiation exposures compared to past missions, is increasing. Until now, the physical doses of space radiation have been measured, but measurement of direct biological effects has been hampered by its low dose and low dose-rate effect. To assess the biological effects of space radiation, we launched and kept frozen mouse embryonic stem (ES) cells in minus eighty degree Celsius freezer in ISS (MELFI) on the International Space Station (ISS) for a maximum of 1,584 days. The passive dosimeter for life science experiments in space (PADLES) was attached on the surface of the sample case of the ES cells. The physical dosimeter measured the absorbed dose in water. After return, the frozen cells were thawed and cultured and their chromosome aberrations were analyzed. Comparative experiments with proton and iron ion irradiation were performed at particle accelerators on Earth. The *wild-type* ES cells showed no differences in chromosomal aberrations between the ground control and ISS exposures. However, we detected an increase of chromosome aberrations in radio-sensitized histone H2AX heterozygous-deficient mouse ES cells and found that the rate of increase against the absorbed dose was 1.54-fold of proton irradiation at an accelerator. On the other hand, we estimated the quality factor of space radiation as 1.48 ± 0.2. using formulas of International Commission of Radiation Protection (ICRP) 60. The relative biological effectiveness (RBE) observed from our experiments (1.54-fold of proton) was almost equal (1.04-fold) to the physical estimation (1.48 ± 0.2). It should be important to clarify the relation between biological effect and physical estimates of space radiation. This comparative study paves a way to reveal the complex radiation environments to reduce the uncertainty for risk assessment of human stay in space.

1. Introduction

In space, various types of radiation exist. In particular, the galactic cosmic rays (GCRs), which contain high charge and energy (HZE) particles, the solar energetic particles (SEPs), which mainly contain high energy protons from solar activity, and the trapped particles of protons and electrons in the magnetic field of the Earth are significant (Durante et al., 2008; Goodhead, 2018). Space radiation and microgravity is a limiting factor for manned exploration in the Moon of the earth and Mars. Though intensive studies have been carried out using animal cells and individual animals as cancer model to clarify the effect of space radiation, most of the experiments were done on the ground as simulation using

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X-ray, gamma-ray or heavy ions at accelerators. So, the results inevitably included the uncertainty comparing with the real space radiation. Several space experiments using frozen mammalian cells were performed. The increase of mutation or DNA damages were detected, but they did not lead to quantitative analyses because of low dose of space radiation (Ohnishi 2016). The chromosome aberration analyses of the astronauts were performed to investigate the DNA damage responses around the flight time on the ISS, revealing the personal difference of pre- and after flight responses and risk of cancers (Cucinotta et al., 2008; George et al., 2013). In 2019, the monozygotic twin astronauts were compared with the effects of 1-year stay on the ISS and on the ground (Garrett-Bakelman et al., 2019). On the ISS, the astronaut had a physical dose of 76.18 mGy and dose equivalent of 146.34 mSv (quality factor = 1.92). The chromosome aberration analyses revealed increased inversions and translocations compared to that on the ground. However, the results were not statistically significant. In 2020, the comprehensive multi-omic analyses revealed unique results of mitochondrial stress by spaceflight effects (Silveira et al., 2020). Our study was for the first time showed the biological effects of space radiation quantitatively, by using radiation sensitized histone H2AX-deficient mouse ES cells and for more than 4 years on the ISS in frozen state. So far, physical measurements of galactic cosmic ray (GCR) were analyzed (Simpson, 1983; Mewaldt, 1994; Berger et al., 2020). Nagamatsu et al. (2013) measured the absorbed dose rate (in water) of space radiation in the Japanese experimental module "Kibo" using PADLES (TLD and CD-39) detector (Nagamatsu et al., 2000). The average absorbed dose rate was 0.319 mGy/day from 12 locations of the Japanese module "Kibo" during the time of June 1, 2008 ~ March 29, 2009 at the time between cycle 23 and cycle 24 of the solar cycle. During Mars exploration, the Radiation Assessment Detector (RAD) dosimeter mounted on the rover Curiosity detected the absorbed dose rate of 0.210 mGy/day on the surface of Mars and 0.464 mGy/day during the transit (Zeitlin et al., 2013). From these physical measurements, the risk on living organisms (such as cell-death, organ failure, chromosome aberration, mutation, cancer) can be estimated based on the quality factor <Q> defined by the ICRP60 (ICRP, 1991) as a dose equivalent (Sv). The quality factor (Q) of radiation in water was specified depending on the linear energy transfer (LET; L) by ICRP60 (1991) as formulas of $Q(L) = 1$ ($L \leq 10$ keV/μm), $Q(L) = 1 + 0.32L\text{-}2.2$($10 \leq L \leq 100$ keV/μm), $Q(L) = 300/L^{1/2}$ ($\geq$100 keV/μm).

However, space radiation is composed of many kinds of particles with different energies and astronauts are continuously irradiated with low dose rates. As such, the space environment cannot be precisely reproduced on the ground, including potential interactions of many kinds of radiations and interactions with microgravity. Thus, the risk from space radiation on living organisms are difficult to evaluate experimentally with ground-based analogs. Our experiment was absolutely different from the other animal model attempt to quantify RBE. We did the direct quantitative measurement of biological effect of space radiation on the ISS. Our purpose is to determine whether the real biological effect is the same as ground-based experiment using ICRP60 quality factors or not in order to exclude the uncertainty of risk assessment. In our experiment we analyzed chromosome aberrations using fluorescent in situ hybridization (FISH) method (Gall and Pardue, 1969) that is known as golden standard related to carcinogenesis. We quantified the biological effects of space radiation using frozen mouse cells in space and compare with physical estimates, which would contribute to the reduction of the uncertainty of risk evaluation of human space flights (Yoshida et al., 2010).

2. Materials and methods

2.1. Mouse ES cells

To produce histone H2AX gene-deficient mouse ES cells, male and female histone H2AX heterologous-deficient mice were mated. The 2-cell embryos were collected from superovulated mice at 1.5 day of pregnancy and cultured in KSOM medium (EmbryoMax®, Merck Millipore, Germany) for 2.5 days. Expanded blastocysts were cultured in the Knockout DMEM supplemented with 20% Knockout Serum Replacement (KSR, Life technologies, USA), 0.2mM L-glutamine (Thermo Fisher Scientific, Waltham, USA), 0.1mM nonessential amino acids (Thermo Fisher Scientific), 1000 U/ml ESGRO (Merck Millipore), and 0.1 mM 2-mercaptoethanol (Thermo Fisher Scientific) for 7 days. Founder colonies were isolated and cultured on mouse embryonic fibroblast (MEF) feeder cells in 24-well plates, and evaluated the genotypes by PCR using H2AX-K14 (5′-CCAGGTGGGCTTGTAGCFCTC-3′) and H2AX-PCR2 (5′-GGATCTTCGTGGATTACATAGCC-3′) for H2AX-deficient alleles with a product of about 400 bp, and H2AX-PCR2 and H2AX-PCR3 (5′-GAGACTCTTACCGGCCTGTGGAC-3′) for H2AX *wild-type* alleles with a product of about 450 bp. *Wild-type*, heterozygous-deficient ES cells were selected and used for analyses.

All studies utilizing mice were approved by the Osaka City University (Graduate School of Medicine) Animal Care and Use Committee by Prof. Ogura H. as a chief board under Protocols 08063 and 10020. According to relevant ethical regulation of Osaka City University (Graduate School of Medicine) Animal Care and Use Committee, the animal studies were performed. This study is reported in accordance with ARRIVE guidelines.

2.2. Preparation of space samples

After culturing *wild-type* and histone H2AX gene-deficient mouse ES cells, the cells were suspended in a cell cryopreservation solution, CELLBANKER (Zenoaq Resource, Japan), dispensed into a cryotube at a concentration of 2×10^6 cells/tube, and cryopreserved (−150 °C). These tubes were launched on March 2, 2013 as a "Stem Cells" project from the NASA Kennedy Space Center in a freezer aboard the SpaceX-2 Dragon. After arriving at the ISS, cells were stored in a minus eighty degree Celsius laboratory freezer (MELFI) in the Japanese experiment module "Kibo". The measured temperature in the MELFI was about −95 °C. A physical dosimeters, PADLES were attached to the package of cell samples.

2.3. Irradiation by accelerator

Using an accelerator, the Heavy Ion Medical Accelerator in Chiba (HIMAC) at NIRS, mouse ES cells were irradiated by proton and Fe-ion particle beams in a frozen state on dry ice as references of single beam irradiation. The proton beam had a kinetic energy of 230 MeV and the LET was 0.415 keV/μm. The Fe-ion beam was 500 MeV/n and the LET was 218 keV/μm.

2.4. Chromosome analyses

The cells cryopreserved and stored on the ground or on the ISS were thawed at 37 °C and suspended in KSR medium for evaluation of cell survival. The ES cells were cultured in KSR medium for 17 h at 37 °C in a CO_2 incubator. Then, Colcemid (KaryoMAX, Thermo Fisher Scientific, Waltham, USA) was added to halt the cells in metaphase and after 4.5 h of incubation, Calyculin A (Fuji-Wako, Tokyo, Japan) was added and cultured for 30 min to condense the G_2-phase chromosomes (Premature chromosome condensation; PCC) (Gotoh, 2009; Kawata et al., 2004). After treatment with 0.075 M KCl at 37 °C for 30 min, cells were fixed with Carnoir fixative and spread onto glass slides. The chromosomes were analyzed by three-color FISH method. The FISH probes (Metasystems, Oberkochen, Germany) were hybridized (chromosome 1 was stained with FITC, chromosome 2 was stained with Spectrum Orange, and chromosome 4 was stained yellow using a 1:1 combination of FITC and Spectrum Orange). Chromosome spread was counterstained with 4, 6-diamidino-2-phenylindole (DAPI). Analysis was performed using Cytovision software (LEICA Microsystems, Wetzlar, Germany). The chromosome aberrations were classified as normal, apparently simple translocation (AST), complex translocation (COM), incomplete

translocation (IT), dicentric (DIC), and incomplete dicentric (ID) (George et al., 2015; Hada et al., 2007).

2.5. Statistical analyses

The frequency of chromosome aberrations was analyzed from several glass slides made from the same sample of cells. The frequency of chromosomal aberrations observed as painted chromosomes was calculated as the ratio between aberrations counted and total cell numbers analyzed. On average, about 1,000 cells were analyzed. For Fe-ion irradiated cells, about 500 samples were examined, because enough aberration samples were found to calculate the frequency. The exchange frequencies in individual chromosomes were extrapolated to whole-genome equivalents using a modified version of the method developed (Lucas et al., 1992). Standard errors for aberration frequencies were estimated assuming Poisson statistics. Error bars in each figure show SEs of the mean values. A linear regression model was used to fit dose-responses for chromosome aberrations of low doses. Using the least square method, the linear coefficients were calculated as slopes with Correlation coefficients (R^2) in Figure 2. The y-intercepts were not fixed to 0.

3. Results

3.1. Launching of mouse ES cells to ISS and physical dosimetry

The frozen mouse ES cells were launched to the ISS and stored for long periods of time (with a maximum of 1,584 days) in the MELFI freezer to analyze chromosome aberrations (Yoshida et al., 2010). In this experiment, *wild-type* and histone H2AX gene heterozygous-deficient ES cells were used. It is because histone H2AX is involved in DNA double-strand break repair, and defects in this gene have been reported to increase radiosensitivity (Bassing et al., 2002; Yoshida and Morita, 2004; Franco et al., 2006). The cell samples were stored for periods of 443 days, 711 days, 1,167 days, and 1,584 days. The PADLES dosimeter was attached on the surface of the ES cell sample case containing 25 tubes. Two cases were packaged by paper and stocked in MELFI (Morita et al., 2021). The PADLES used a CR-39 plastic nuclear track detector and thermoluminescent dosimeter (TLD). PADLES measured the absorbed dose including the influence of all particles of primary and secondary radiation from GCR, SEPs and trapped radiation received during the stock time in the ISS. Particles above 10 keV/μm were measured by CR-39, and those below 10 keV/μm were measured by TLD. The database of fading and aging of CR-39 and TLD in the installation temperature (−80 to 60 °C) of the PADLES was obtained from the ground irradiation test using the heavy particle accelerator. The correction was applied according to the installation temperature history in our experiments. The details of distributions of the flux of particles detected by PADLES are shown (Morita et al., 2021). The total absorbed dose in water of radiation during each period was 174.4 ± 15.9 mGy (443 days), 289.1 ± 18.4 mGy (711 days), 412.9 ± 22.2 mGy (1,167 days), and 560.05 ± 59.0 mGy (1,584 days), respectively (Table 1). The absorbed dose for 1,584 days showed that particles with low LET of 10 keV/μm or less gave 537.1 ± 59.1 mGy (95.9%), while those with high LET more than 10 keV/μm gave 22.95 ± 3.11 mGy (4.10%). The dose equivalents that showed the biological effectiveness considering quality of irradiation source were calculated by application of the LET dependent quality factor formulas (ICRP60, 1991). The total dose equivalent was 830.4 ± 74 mSv and the dose equivalent of high LET particles (>10 keV/μm) was 293.25 ± 44.7 mSv. So, high LET particles having only 4.10% of the total absorbed dose have possibly 35.3% of biological effect. We suppose that large (several or more cells) number of cells in the track of radiation will be impacted by the high dose rate events, while the low LET particles may have a chance to hit to a single cell. The absorbed dose rate from data of 1,584 days was 0.355 ± 0.04 mGy/day and the dose equivalent rate was 0.525 ± 0.05 mSv/day, indicating that the quality factor of space radiation in MELFI on the ISS was 1.48 ± 0.2.

3.2. Chromosome aberrations of ES cells from ISS and ground control

The frozen *wild-type* and histone H2AX heterozygous-deficient ES cells were retrieved from the ISS, thawed and cultured for 22 h on the ground along with the frozen cell samples stored at JAXA (Ibaragi, Japan) as the ground controls designated backup (BU). The chromosomes of cells were analyzed by FISH. The results are shown in Table 2. For *wild-type* ES cells stored on the ISS as well as BU on the ground, the rates of total chromosome aberrations from 443 days to 1,584 days were almost at basal levels. Thus, an increase in chromosome aberration due to the number of storage days on the ISS was not detected (Figure 1). In contrast, in histone H2AX gene heterozygous-deficient ES cells stored on the ISS, increases in the rates of chromosome aberrations were observed, while in the same cells on the ground (BU) were not (Figure 1). Since DNA repair was thought not to occur in the cells while they were frozen, DNA damage induced by space radiation would be accumulated equally both in *wild-type* cells and histone H2AX gene heterozygous-deficient cells. As the chromosome aberrations were formed during the culture of cells after thawing on the ground, their difference in yield showed the capability of the repair process of each cell type of H2AX gene. In other words, the *wild-type* cells could repair all DNA damage normally in post-thaw culture, but the H2AX heterozygous-deficient cells were unable to fully repair damage, resulting in abnormal chromosomes. These results, did not indicate that normal man could repair all damage accumulated for more than 4 years within a day and he is unaffected, while histone H2AX gene heterozygous-deficient man would be affected. This was an experiment using frozen cells, and not living bodies. What was most important was that by using frozen histone H2AX gene heterozygous-deficient cells, we could biologically detect accumulated chronic damages by low-dose, and low dose-rate space radiation as acute effects of chromosome aberrations. Then we could compare them to the effects of

Table 1. Physical measurement of the space radiation in MELFI on the ISS by Bio PADLES dosimeters.

Bio PADLES data	443 days #1, #6 (Av)	711 days #3	1167 days #4	1584 days #2, #5 (Av)
Total Absorbed Dose [mGy in water]	174.4 ± 15.9	289.1 ± 18.4	412.9 ± 22.2	560.05 ± 59.0
Total Dose Equivalent [mSv]*	247.15 ± 26.3	374.1 ± 21.1	575.2 ± 31.9	830.4 ± 74.0
Absorbed Dose Rate [mGy/day]	0.39 ± 0.03	0.41 ± 0.03	0.35 ± 0.02	0.355 ± 0.04
Dose Equivalent Rate [mSv/day]*	0.6 ± 0.06	0.53 ± 0.03	0.49 ± 0.03	0.525 ± 0.05
Absorbed Dose (≤10keV/um)[mGy]	164.3 ± 16.0	280.4 ± 18.4	398.5 ± 22.3	537.1 ± 59.1
Absorbed Dose (>10keV/um)[mGy]	10.1 ± 1.39	8.64 ± 0.77	14.4 ± 1.5	22.95 ± 3.11
Dose Equivalent (>10keV/um) [mSv]*	100.9 ± 20.9	93.7 ± 10.3	176.7 ± 22.8	293.25 ± 44.7
Mean QF	1.52 ± 0.20	1.29 ± 0.11	1.39 ± 0.11	1.48 ± 0.2

From the launch on March 2, 2013 to July 3, 2017, the mouse ES cell samples were collected in four batches with PADLES dosimeters in MELFI freezer on the ISS. The measured values shown here are from the PADLES dosimeter installed at the time of the first launch. The values calculated as average from two data are shown as Av.

* Calculated using quality factors defined by ICRP60.

standard proton beams obtained on the ground by accelerator, using the same type of cells and the same method.

3.3. Chromosome aberrations by ion beams

Using an accelerator, the histone H2AX gene heterozygous-deficient ES cells were irradiated by protons and Fe-ion particle beams in a frozen state as references of single beam irradiation. In our experiment, we used proton of high energy of 230MeV/n and very low LET (0.415 keV/μm). The quality factor of the high energy proton is defined as 1 in ICRP60 and the recent comparative data indicated that quality factor of both high energy proton and gamma-ray was the same as low LET radiations (Willers et al., 2018; Kowalska et al., 2019). The Fe ion was used as reference of high LET particle beam (500 MeV/n, LET 218 keV/μm). The results are shown in Table 2 and Figure 2. When the rate of chromosome aberrations by proton and Fe-ion beam (both dose rates; approximately 300 mGy/min) was plotted on the axis of absorbed doses (Gy), it became clear that the chromosome aberrations increased depending on the absorbed doses (Figure 2). The rates of chromosome aberration of ISS-stocked ES cells were corrected by subtracting associated ground-based aberration values (BU) as control. The physical dose of ISS in MELFI were also subtracted ground-based aberration as controls. Also, the rates of chromosome aberration of the proton and Fe ion irradiations were subtracted from non-irradiated values. The values of chromosome aberration were plotted against the absorbed doses as shown in Table1 Then, the regression lines were drawn. Because the chromosome aberration rates were subtracted respective control values, the y-intercepts were not fixed at 0 (Figure 2) to gain the slopes of lines. The coefficients of the slopes for Fe-ion beam, proton beam, and space radiation in the ISS (MELFI) were 0.0667 ± 0.0086, 0.0085 ± 0.0022, and 0.0131 ± 0.0030, respectively, as shown in Figure 2. We indicated the R^2 coefficient of determination showing the goodness of fit of the regression prediction. The R^2 value for Fe was 0.96, 0.86 for ISS (MELFI) and 0.88 for proton, respectively. Each slope was thought to reflect the relative biological effectiveness of the radiation source, such as quality factor <Q> for specified irradiation type. Thus, the RBE surrogating a quality factor of space radiation in the ISS (MELFI) was calculated to be 1.54-fold that of proton beam regarded as <Q>=1.0. The slope of the Fe beam was found to be 7.85-fold that of proton beam.

In Table 2, types of chromosome aberrations are shown. They were classified as normal, apparently simple translocation (AST), complex translocation (COM), incomplete translocation (IT), dicentric (DIC), and incomplete dicentric (ID). The aberration of AST is most popular chromosome aberrations having apparently simple translocation of the chromosome. The incomplete translocation is also single exchange, but chromosome identification is not possible because of restriction of FISH probes. These chromosome translocations are stably transmitted through the cell division. The complex chromosome aberration having more than two translocations is thought to occur by high LET particles and unstable. The dicentrics and incomplete dicentrics are also unstable chromosome aberration. As we made chromosome samples within one cycle of cells, we should be able to observe even unstable chromosome aberration such as dicentric and complex type of aberration.

The chromosomal aberrations in the histone H2AX heterozygous-deficient cells caused by 0.5 Gy (near the absorbed dose for 1,584 days exposure by space radiation) proton beam included AST (12.5%), ID (12.5%), and many of IT (75%). They did not show COM that had more than two translocations. The 0.5 Gy of Fe ion beam induced a relatively large amount of COM (29%) in addition to IT (52%). The histone H2AX heterozygous-deficient ES cells in MELFI on the ISS exposed to 0.56 Gy of space radiation during 1,584 days revealed aberrations with many IT (75%) and AST (25%), but no COM, indicating qualitative similarity to the results by proton irradiation rather than Fe ions.

Table 2. Chromosomal aberration rates in *wild-type* and histone H2AX gene heterozygous-deficient mouse ES cells.

ES cells		Days	Total spread scored	Apparently simple translocation	Complex translocation	Incomplete translocation	Dicentric	Incomplete dicentric	Total
H2AX +/+	BU	443	1018	0	0	0	0	0.001 ± 0.001	0.001 ± 0.001
		711	1002	0	0	0	0	0.001 ± 0.001	0.001 ± 0.001
		1167	1049	0	0	0	0	0	0
		1584	1027	0	0	0	0	0	0
	ISS	443	1004	0.001 ± 0.001	0	0	0	0	0.001 ± 0.001
		711	1013	0	0	0.001 ± 0.001	0	0	0.001 ± 0.001
		1167	1295	0	0	0	0	0	0
		1584	1016	0	0	0.001 ± 0.001	0	0	0.001 ± 0.001
H2AX +/-	BU	443	1240	0	0	0	0	0.001 ± 0.001	0.001 ± 0.001
		711	1253	0	0.001 ± 0.001	0	0	0.001 ± 0.001	0.002 ± 0.001
		1167	1290	0.001 ± 0.001	0	0	0	0	0.001 ± 0.001
		1584	1032	0	0	0	0	0.001 ± 0.001	0.001 ± 0.001
	ISS	443	1042	0	0.001 ± 0.001	0	0	0.001 ± 0.001	0.002 ± 0.001
		711	1277	0.002 ± 0.001	0	0	0	0.001 ± 0.001	0.003 ± 0.002
		1167	1205	0	0	0.002 ± 0.001	0.001 ± 0.001	0.003 ± 0.002	0.006 ± 0.002
		1584	1060	0.002 ± 0.001	0	0.006 ± 0.002	0.001 ± 0.001	0	0.008 ± 0.003
H2AX +/-	Control	0 Gy	1017	0	0	0	0	0.001 ± 0.001	0.001 ± 0.001
	HIMAC Proton	0.2 Gy	1725	0.001 ± 0.001	0	0.002 ± 0.001	0	0.002 ± 0.001	0.005 ± 0.002
		0.5 Gy	866	0.001 ± 0.001	0	0.006 ± 0.003	0	0.001 ± 0.001	0.008 ± 0.003
		1.0 Gy	594	0	0.002 ± 0.002	0.005 ± 0.003	0	0.003 ± 0.002	0.010 ± 0.004
	HIMAC Fe-ion	0.2 Gy	1064	0.003 ± 0.002	0.001 ± 0.001	0.003 ± 0.002	0	0.004 ± 0.002	0.010 ± 0.003
		0.5 Gy	595	0.003 ± 0.002	0.012 ± 0.004	0.022 ± 0.006	0	0.005 ± 0.003	0.042 ± 0.008
		1.0 Gy	586	0.009 ± 0.004	0.026 ± 0.007	0.020 ± 0.006	0.003 ± 0.002	0.007 ± 0.003	0.065 ± 0.011

Chromosome aberrations were analyzed by FISH. Chromosomes were classified as normal, complex translocation (COM), apparently simple translocation (AST), incomplete translocation (IT), dicentric (DIC), and incomplete dicentric (ID). The rates of chromosome exchanges were extrapolated to whole genome equivalents as described in Methods. BU indicates the results from mouse ES cells as backup controls stocked on the ground. ISS indicates the results from mouse ES cells stocked on the ISS for indicated periods.

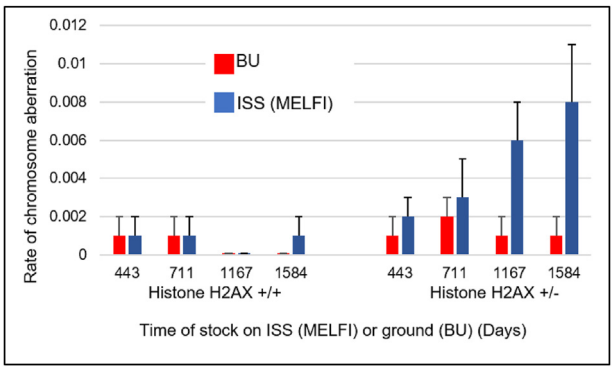

Figure 1. Comparison of the incidence of chromosomal aberrations of *wild-type* and histone H2AX gene heterozygous-deficient ES cells on the ground (BU) and on the ISS (MELFI). The frozen *wild-type* and histone H2AX gene heterozygous-deficient ES cells stocked on the ground (BU) and in MELFI freezer on the ISS were thawed, cultured on the ground and the chromosomes were analyzed by FISH method. The frequencies of chromosome aberrations were extrapolated to whole genome equivalents as described in Methods.

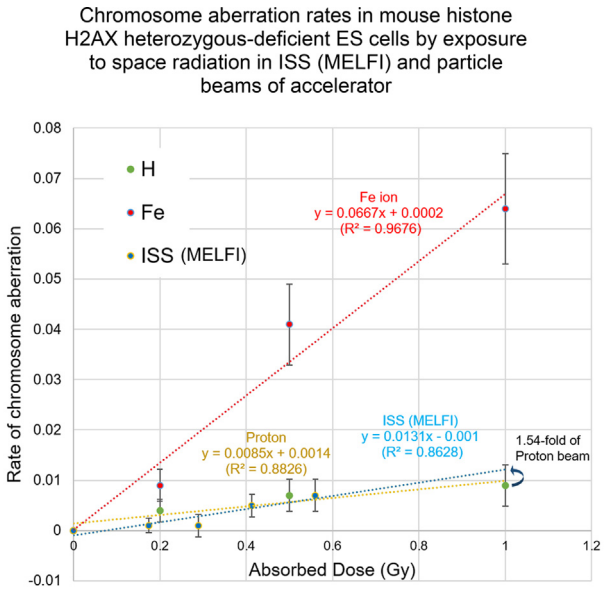

Figure 2. Relationship between absorbed dose and chromosomal aberration rate of histone H2AX gene heterozygous-deficient ES cells. The vertical axis represents the rates of chromosomal aberrations and the horizontal axis represents the absorbed doses. Chromosome aberrations of histone H2AX gene heterozygous-deficient ES cells by Fe-ion beam (500 MeV/n, LET; 218 keV/μm) and proton beam (230 MeV, LET; 0.415 keV/μm) were plotted. Chromosome aberrations of ISS-stocked (in MELFI freezer) ES cells subtracted those on the ground were plotted against their absorbed doses measured by sample-attached PADLES dosimeters. The slope of the linear approximation line reflects the biological effects of each source of radiation as quality factor <Q>.

3.4. Comparison of quality factors

In this study, chronically accumulated DNA damage to frozen mouse histone H2AX heterozygous-deficient ES cells was measured as acute effects. As shown in Figure 2, assuming that the low LET proton (LET = 0.415 keV/μm) was defined as a unit of the quality factor $< Q > = 1$ (ICRP60), the RBE in MELFI on the ISS was estimated to be 1.54 acting as a surrogate for quality factor of space radiation. This value was almost equal or slightly higher (+4%) than the quality factor of 1.48 ± 0.2 derived from PADLES data (1,584 days) and ICRP60 formulas. Thus, the biological effect of space radiation in MELFI on the ISS from our result was not significantly different from expectation by physical estimates.

4. Discussion

We launched the mouse ES cells from the ground to the ISS and after storage in the MELFI freezer, they were retrieved to the ground. So the influence of the stress of high gravity of launching and return, microgravity on ISS and magnetosphere should be considered. As the histone H2AX heterozygous-deficient mouse ES cells on the ISS for the shortest period of stock of 443 days showed chromosome aberration frequency as 0.002 + 0.001, while the same cells on the ground showed 0.001 + 0.001. Since the difference was small and the frozen cells are thought to not be affected by gravity, the effect of gravity would not be significant. We note that possibility of effects of stresses of gravity or magnetic fields have not been suggested in prior studies of chromosomal aberrations.

In our study, we measured the biological effect of space radiation to the ES cells in MELFI on the ISS as 1.54-fold that of the proton beam. On the other hand, the physical dosimeter PADLES kept in MELFI showed the quality factor as 1.48 for 1,584 days sample. So, we concluded that the biologically measured quality factor was 1.04-fold of the physical estimate using ICRP60 quality factors. The value of the quality factor as 1.54 in MELFI was lower than those measured in many points in the ISS. The quality factor in the "Kibo" module measured by Nagamatsu et al. were 1.95–2.22. The ES cells were contained in MELFI freezer, so that the position of the MELFI on the ISS or MELFI's aluminum wall may lower the quality factor. Though the quality factor in MELFI was low, the stable environment in MELFI might be better to the comparative study of biological and physical effect of space radiation for more than 4 years.

We applied our comparative result of biological and physical estimates of space radiation from MELFI to the living area in the ISS. The average absorbed dose rate from 12 monitoring points of living area in the "Kibo" module was 0.319 mGy/day (June 1. 2008∼ March 29, 2009) (Nagamatsu et al., 2013) and the averaged quality factor was calculated as 1.95 defined by ICRP60. From the data, the averaged absorbed dose rate was 0.319 mGy/day in "Kibo" module as it was. But the quality factor must be corrected 1.04-fold to reflect the real biological effectiveness from this study. Then, corrected quality factor would become 1.95 × 1.04 = 2.03. Then the averaged dose equivalent rate in "Kibo" would become 0.319 mGy/day x 2.03 = 0.648 mSv/day. The result was very close to and consistent with the physical estimate by Nagamatsu et al. (2013) before (0.618 + 0.102 mSv/day).

Our result estimated 4% increase of biological effect comparing with the PADLES physical estimate. On the other hand, the biological effectiveness of high LET particle, Fe-ion (LET = 218 keV/μm) was estimated to be 7.85-fold that of proton in this experiment. It is much lower than the value of <Q>=20.3 calculated by ICRP60 formula. The biological effectiveness of radiation and quality factor may deviate for high LET like Fe-ions (>200 keV/μm) depending on the biological endpoint (Cacao et al., 2016). Our result suggests that ICRP60 formulas may possibly overestimate the biological effects of higher LET particles when we consider the risks of humans in deep space. In addition, it may be necessary to reconsider the dependence of the quality factor on the particle track structure (Goodhead, 2018; Cucinotta et al., 2015a; Cucinotta, 2015b) as introduced by Cucinotta and used as NASA (Cucinotta et al., 2013; Cucinotta et al., 2020). The quality factor, QF_{NASA} is established to fit a large number of experimental results based on a track structure model, and is used for cancer risk predictions (Goodhead, 2018; Cucinotta et al., 2015a, 2017). The space radiation QF developed from track structure concepts by Cucinotta allows for an uncertainty assessment which is not possible in the ICRP approach. The weighted average of the NASA QF for solid cancer and leukemia are very similar to the ICRP 60 average QF as shown by Cucinotta et al. (2013). In the case of leukemia as endpoint, the QF of the high LET particles about 200 keV/μm was estimated to be about 9.

In this study, we measured the accumulated effect of space radiation as acute one. However, in human space exploration, astronauts are exposed to space radiation at low-dose rates for long periods of time. As the human cells have DNA repair and apoptosis systems, the effects of space radiation should be reduced by dose and dose-rate effectiveness factors (DDREF). By ICRP60, the value of DDREF is recommended as 2, meaning a 50% reduction of effects by low dose-rate irradiation compared with acute effects. In experiments on mouse tumor induction, DDREF values were found to be from 1 to >4 (Cucinotta et al., 2017; Tanaka et al., 2013). However, the DDREF used in radiation protection is for gamma-rays. The measurements in space may provide information on dose-rate modification for space radiation, and possibly decrease the risk assessment of space radiation.

The method described here may be applied to other space environments. The comparisons of similarity and differences of our QF estimates in relation to those used in radiation protection on Earth based on ICRP recommendations suggest the importance of flight radiobiology experiments. For future work, we think it should be better to use frozen H2AX-heterozygous deficient human ES cells rather than mouse ES cells. The human cells are much suited for human risk assessment and easier to analyze chromosome aberration because of their variety of lengths. Furthermore, to elucidate the dose and dose-rate effect factors in space, we must launch individual mice or experimental animals to analyze the chromosome aberrations in space. Such experiments in deep space as on the Moon's surface can contribute to reduce the uncertainty of the risk assessment of human prolonged journeys and stay in space.

Declarations

Author contribution statement

Kayo Yoshida; Megumi Hada; Kiyomi Eguchi-Kasai; Aiko Nagamatsu; Takashi Morita: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents materials, analysis tools or data; Wrote the paper.

Akane Kizu; Kohei Kitada: Performed the experiments; Analyzed and interpreted the data.

Sachiko Yano; Hiromi Hashizume Suzuki; Toshiaki Kokubo: Performed the experiments; Contributed reagents materials, analysis tools or data.

Takeshi Teramura; Hitomi Watanabe; Gen Kondoh: Performed the experiments; Analyzed and interpreted the data; Contributed reagents materials, analysis tools or data.

Premkumar Saganti: Analyzed and interpreted the data; Wrote the paper.

Francis A. Cucinotta: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at https://doi.org/10.1016/j.heliyon.2022.e10266.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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