J. RADIAT. RES., 43: SUPPL., S133–S136 (2002)

Detection of DNA Damage Induced by Space Radiation in Mir and Space Shuttle

TAKEO OHNISHI[1]*, KEN OHNISHI[1], AKIHISA TAKAHASHI[1], YOSHITAKA TANIGUCHI[2], MASARU SATO[3], TAMOTSU NAKANO[3] and SHUNJI NAGAOKA[4]

Space flight / DNA strand break / Flight period

Although physical monitoring of space radiation has been accomplished, we aim to measure exact DNA damage as caused by space radiation. If DNA damage is caused by space radiation, we can detect DNA damage dependent on the length of the space flight periods by using post-labeling methods. To detect DNA damage caused by space radiation, we placed fixed human cervical carcinoma (HeLa) cells in the Russian Mir space station for 40 days and in an American space shuttle for 9 days. After landing, we labeled space-radiation-induced DNA strand breaks by enzymatic incorporation of [^{3}H]-dATP with terminal deoxyribo-nucleotidyl transferase (TdT). We detected DNA damage as many grains on fixed silver emulsion resulting from β-rays emitted from ^{3}H-atoms in the nuclei of the cells placed in the Mir-station (J/Mir mission, STS-89), but detected hardly any in the ground control sample. In the space shuttle samples (S/MM-8), the number of cells having many grains was lower than that in the J/Mir mission samples. These results suggest that DNA damage is caused by space radiation and that it is dependent on the length of the space flight.

INTRODUCTION

The major component of space radiation (more than 90%, in general) is protons, and only neutrons and heavy particles such as Fe and so on are regarded as high linear energy transfer (LET) radiation. Large amounts of radiation penetrate into spacecraft, where they have been measured physically. The intensity of space radiation is known to be dependent on solar cycle and short-term eruptions. In the past several years, physical monitoring of space radiation has detected about 1 mSv per day[1,2)], which exceeds the average level of radiation received on earth by more than one hundred times. Therefore, it is important to use biological dosimetry to calculate the relative biological effectiveness (RBE) with respect to human health. It is assumed that such space radiation might induce more DNA damage than the radiation on the ground, because the earth is protected from space radiation by the magnetic field of the earth. Here we tried to directly measure the DNA damage by post-labeling of the 3'-ends of DNA molecules with strand breaks caused by space radiation.

*Corresponding author: Phone: +81–744–22–3051 ext. 2264
Fax: +81–744–25–3345
E-mail: tohnishi@naramed-u.ac.jp

[1] Department of Biology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara, 634–8521

[2] Toray Research Center Inc., Tebiro Kamakura, Kanagawa, 248–8555

[3] Centrifuge Project Team, NASDA, Tsukuba, 305–8505

[4] Department of Gravitational Physiology, Fujita Health University School of Health Sciences, Aichi, 470–1192, Japan

MATERIALS AND METHODS

Samples

A cell line of human cervical carcinoma, HeLa (provided by JCRB, Tokyo, Japan), was cultured at 37°C in Delbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, penicillin (50 U/ml), streptomycin (50 μg/ml) and kanamycin (50 μg/ml). Cells were plated on CR39 plates (17.5 mm × 35 mm) which were placed in 10-cm diameter cell culture dishes. CR39 plates were provided for other physical analyses of space radiation. About 10 hr after the cell plating (about 5×10^5 cells/dish), the cells on the plates were washed with PBS (minus Ca^{2+} and Mg^{2+}) and fixed with methanol for 10 min. In the MIR experiment, the

samples were carried from Nara, Japan, to Baikonur, Kazakhustan at room temperature in the dark, and there they were loaded on a Progress M-35 rocket. The space samples were carried by "Progress" and exposed to space radiation on the Mir Space Station for 40 days (J/Mir mission (STS-89); July 5-August 14, 1997). For the 8th Shuttle/MIR Mission experiment (S/MM8; January 23-Feburary 1, 1998), the samples (16 plates for space and 16 plates for earth control) were similarly carried from Nara Medical University, Japan, to Kennedy Space Center, Florida, USA. The space samples were exposed to space radiation on the Space Shuttle "Endeavor" for 9 days. During this period, the control samples were stored on earth at room temperature in the dark.

In situ enzymatic post-labeling assay

The present study used the TdT post-labeling assay[3,4] to detect DNA strand breaks induced by space radiation. HeLa cells on the CR39 plates were treated with 20 μg/ml RNase (Sigma, Code No. R-4875, St. Louis, MO) at 37°C for 1 hr and then incubated with 30 μl of reaction mixture consisting of 100 mM sodium cacodylate trihydrate, 1 mM $CoCl_2$, 0.2 mM dithiothreitol, 0.2 mM dATP (TOYOBO, Code No. ATP-101, Tokyo, Japan), 18.5 kBq [^{3}H]-dATP (Amersham, TRK633, Buckinghamshire, England), and 10 units of TdT (Takara, Code No. 2230, Otsu, Japan) at 37°C for 2 hr. After the incubation, the cells were washed with 0.1 % Triton X-100 two times, and with methanol once and then they were dried at 45°C. The cells were dipped into autoradiographic emulsion (KONIKA, NR-M2, Tokyo, Japan) at 40–42°C, and developed after 5 or 14 days. Finally, the samples were stained with 2% Giemsa' solution and observed with a light microscope. The number of grains in 100 cells of each CR39 plate was counted.

RESULTS

The typical photograph of post-labeling method

The typical photograph of [^{3}H]-labeled grains in HeLa cells are shown in Fig. 1. In the S/MM8 experiment, although a few cells in space samples have many grains, there was almost no difference between earth samples (Fig. 1a) and space samples (Fig. 1b). In J/Mir mission samples, HeLa cells having many grains were more frequent and clusters of multigrain cells were also more frequently observed in the space samples (Fig. 1d) than in the earth sample (Fig. 1c).

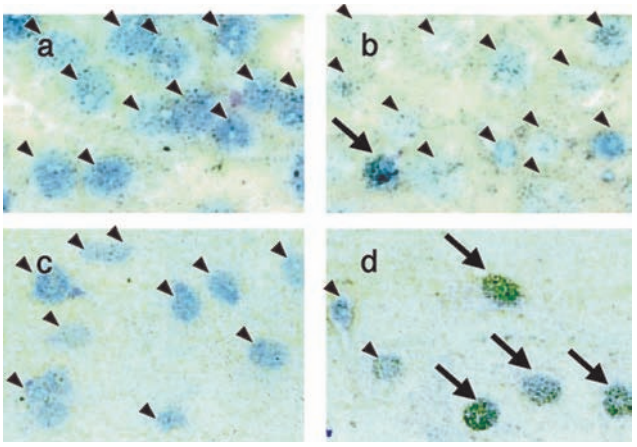

Fig. 1. Visualization of DNA lesions of HeLa cells with *in situ* enzymatic post-labeling assay. The cells on earth (**a**, 9 days experiments and **c**, 40 days experiments) or in space (**b**, 9 days flight and **d**, 40 days flight) were exposed to fixed emulsion for 14 days. Arrows and arrowheads indicate the cells having many grains and few grains, respectively.

Analysis of samples exposed to space for a short and long period

In the S/MM8 experiment, the relationship between grain number per nucleus and cell number having various numbers of grains was similar in the space and earth samples. In J/Mir mission samples, the number of cells having over 21 grains relative to the total cell number was greater in the space sample (about 9 %) than in the earth sample (about 4 %). The ratios of the numbers of cells in space to the number of cells on earth having over 21 grains were calculated for the space and earth samples. As shown in Fig. 2, the ratios were 1.11 and 2.25 for 9-day and 40-day periods of flight, respectively.

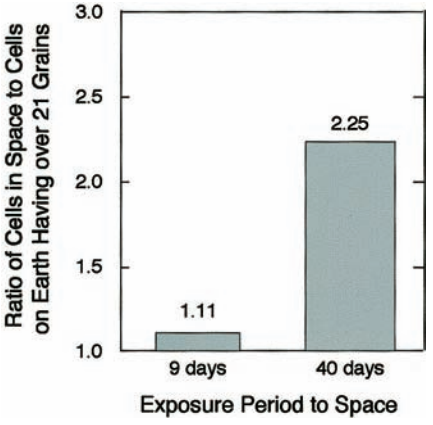

Fig. 2. Ratios of cells in space to cells on earth having over 21 grains in 9 days flight and 40 days flight. [^{3}H]-labeled cells were exposed to fixed emulsion for 5 days.

DISCUSSION

In the present study, we detected DNA damage by the post-labeling method as an exposed grain in fixed silver bromide emulsion more frequently in space samples than in earth samples (Fig. 1). This result shows that space radiation indeed induced DNA damage in space flight samples. Especially, although background noise was included below 20 grains per nucleus, we detected large numbers of nuclei having over 21 grains in samples after space flight for 40 days in the J/Mir mission experiment (Fig. 2). However, we detected a small number of cells having over 21 grains in the samples after space flight for 9 days in S/MM8 when the samples were exposed to the fixed emulsion for the same period (Fig. 2). Since the dose rate of space radiation (about 1 mSv/day[1,2]) and the altitude and inclination of flight orbits were almost the same in the two studies, the variation in results between the two flight experiments is surely due to the difference in flight period. From comparison of the number of cells having over 21 grains between those two space experiments, we found that about 2-fold more DNA damage was induced in the 40-day stay in space than in the 9-day stay. At present, we have no reasonable explanation for the lack of correlation between the ratio of DNA damage (about 2-fold larger in the J/Mir mission) and the ratio of time in space (about 4-fold longer stay in the J/Mir mission (40 days) than in S/MM8 (9 days)). It is possible that the real absorbed doses might have been different in these space flights, because it is different efficiency of shielding levels between Mir and Space Shuttle vehicles. The actual doses of space radiation were not measured with physical dosimeters in the flights.

In J/Mir mission samples, we detected clusters of several cells containing many grains, but we detected no such clusters in ground samples, as shown in Fig. 1. We presume that the clusters result from large tracks of heavy particles present in space radiation. Compared with low-LET radiation such as γ-rays or X-rays, high-LET heavy particles result in larger numbers of DNA strand breaks[5], and thus the clusters observed in J/Mir mission samples may be due to high-LET heavy particles. Another possibility is that groups of secondary particles of the penetrated space radiations may have been produced right over the cells. Further physical analysis is necessary to confirm that the site of damaged DNA and the tracks of space radiation overlap each other using CR39 plates, which can record the tracks of space radiation.

The biological monitoring performed in the present study is available for determination of the maximum permissible period of exposure to space radiation. However, the RBE of space radiation is unknown at present. Chronic exposure at a low dose-rate is a popular expectation for space. Recently, we found that pre-chronic irradiation at a low dose or a low dose-rate induces a radioadaptive response in mice and cultured human cells[6]. Although the radioadaptive phenomenon is generally noticed with low-LET radiation, there is also the latest report that the radioadaptive response can influence the outcome of neutron exposure[7]. It still remains unclear what caused the radioadaptive response. We must consider the effects of space radiation on the health of space crews in long-term flight on the International Space Station. When the RBE is calculated, it will provide guidelines for the physical protection from the serious effects of space radiation.

ACKNOWLEDGEMENTS

This study was funded in part by the “Ground Research for Space Utilization” promoted by NASDA and the Japan Space Forum. RSC Energia, JGC Co also supported it.

REFERENCES

1. Doke, T., Hayashi, T., Kikuchi, J., Hasebe, N., Nagaoka, S., Kato, M. and Badhwar, G. D. (1995) Real time measurement of LET distribution in the IML-2 Space Lab (STS-65). Nucl. Inst. Methods Phys. Res. **A365**: 524.
2. Hayashi, T., Doke, T., Kikuchi, J., Sakaguchi, T., Takeuchi, R., Takashima, T., Kobayashi, M., Terasawa, K., Takahashi, K., Watanabe, A., Kyan, A., Hasebe, N., Kashiwagi, T., Ogura, K., Nagaoka, S., Kato, M., Nakano, T., Takahashi, S., Yamanaka, H., Yamaguchi, K. and Badhwar, G. D. (1996) Measurements of LET distribution and dose equivalent onboard the space shuttle IML-2 (STS-65) and S/MM-4 (STS-79). Biol. Sci. Space **11**: 355–364.
3. Modak, S. P. and Bollum, F. J. (1970) Terminal lens cell differentiation. III. Inhibitor activity of DNA during nuclear degeneration. Exp. Cell. Res. **62**: 421–432.
4. Gavriel, Y., Sherman, Y. and Ben-Sasson, S. A. (1992) Identification of programmed cell death *in situ* via specific labeling of nuclear DNA fragmentation. J. Cell. Biol. **119**: 493–501.
5. Goodhead, D. T. (1994) Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. Int. J. Radiat. Biol. **65**: 7–17.
6. Takahashi, A. (2002) Pre-irradiation at a low dose-rate blunted p53 response. J. Radiat. Res. **43**: 1–9.
7. Gajendiran, N., Tanaka, K., Kumaravel, T. S. and Kamada N. (2001) Neutron-induced adaptive response studied in Go human lymphocytes using the comet assay. J. Radiat. Res. **42**: 91–101.

Received on May 30, 2002
Revision on August 20, 2002
Accepted on November 1, 2002